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CELIAC DISEASE: COMPLICATIONS AND THE ROLE OF INFECTION IN PATHOGENESIS

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We exist as opposed to
a customary state of not being
in this world of breathtaking exceptions.

-AW

To my family

ABSTRACT

Background: Celiac disease (CD) is an autoimmune disorder occurring worldwide with a prevalence of about 1% of the Western population. The classic presentation comprises symptoms of malabsorption such as diarrhea and weight loss, but the spectrum of symptoms is wide, including asymptomatic disease. CD is induced by dietary gluten in genetically susceptible individuals. The pathogenesis of CD has not been fully elucidated, and although environmental factors such as infant feeding practice and infectious disease have been suggested, results are inconclusive. Treatment of CD consists of a life-long gluten-free diet (GFD). Individuals with CD suffer increased risk of a number of comorbid conditions, including diabetes mellitus type 1, depression and certain malignancies.

Aims: The aim of this thesis was to investigate the risk of venous thromboembolism (VTE), end-stage renal disease (ESRD), and IgA nephropathy (IgAN) in individuals with CD. These studies were carried out with the objective to obtain increased knowledge regarding CD characteristics, in order to optimally design the care of individuals with CD and identify possible groups with increased risk of CD. A separate aim of this thesis was to further investigate the pathogenesis of CD, by assessing the effect of infectious disease at time of gluten introduction in infants on the risk of future CD.

Materials and methods: The risk of VTE was assessed in a cohort of 14,207 individuals with a discharge diagnosis of CD recorded in the Swedish hospital discharge register. When investigating renal complications (ESRD and IgAN), studies were based on a CD cohort identified through Swedish biopsy registers (about 29,000 individuals). Reference individuals, matched for age, sex, calendar period and county, were selected (five per index individual with CD) from the Swedish total population register. Cox regression was used to investigate the associations between CD and outcome data in these population-based cohort studies.

We used the All Babies in Southeast Sweden (ABIS) population-based cohort study, where the parents of all children born in 1997-1999 in the area were invited to participate. Parents of 9,849 children prospectively completed a diary with feeding data and parent-reported infections during the child's first year of life. The pediatric departments in the area reported children diagnosed with CD. Cox regression was used to assess the risk of CD in children with infection at time of gluten introduction.

Results: Among individuals with a discharge diagnosis of CD, 406 (2.6%) suffered subsequent VTE, compared with 1105/76,910 (1.4%) of reference individuals, corresponding to a modestly increased risk of VTE in CD (Hazard ratio, HR, 1.86; 95% Confidence interval, CI, 1.54-2.24). This risk increase was limited to individuals diagnosed with CD in adulthood. Individuals with biopsy-verified CD suffered a three-fold increased risk of future ESRD (HR, 2.87; 95% CI 2.22-3.71). The estimate remained significant in analyses where we adjusted for the presence of type 1 diabetes mellitus, and after restricting the outcome to ESRD recorded in the Swedish patient register *and* in the Swedish renal register. Individuals with biopsy-verified CD also suffered increased risk of future biopsy-verified IgAN (HR, 3.03; 95% CI 1.22-7.56). This risk increase was only seen in men with CD.

Although parent-reported infections were more common among children with CD ($p=0.035$), we found no increased risk of CD among children with any reported infection (HR, 1.8; 95% CI 0.9-3.6) or gastroenteritis (HR, 2.6; 95% CI 0.2-30.8) at time of gluten introduction (analyses adjusted for age at gluten introduction, age at end of breastfeeding, and age at any infection). The majority of Swedish children were breastfed for more than 9 months.

Conclusions: CD is modestly associated with future VTE, likely due to a combination of chronic inflammation and surveillance bias. CD is a risk factor for future ESRD and IgAN. Although absolute risks are low, our findings warrant increased awareness regarding renal function in the care of individuals with CD. Future studies should evaluate the effect of adherence to a gluten-free diet and risk of future renal disease to potentially identify individuals at risk.

Infection at time of gluten introduction does not seem to be a major risk factor for future CD. In a setting where adherence to infant feeding guidelines is high, duration of breastfeeding and age at gluten introduction are no major risk factors for CD. Future studies should include longer follow-up to assess long-term effects of environmental factors during the first year of life.

LIST OF PUBLICATIONS

- I. Ludvigsson JF, Welander A, Lassila R, Ekbom A, Montgomery SM.
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Infectious disease and risk of later celiac disease in childhood.
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LIST OF ABBREVIATIONS

AGA	Gliadin antibodies
CD	Celiac disease
CI	Confidence interval
EMA	Endomysial antibodies
ESRD	End-stage renal disease
GFD	Gluten-free diet
HLA	Human leukocyte antigen
HR	Hazard ratio
IgAN	IgA nephropathy
NPV	Negative predictive value
OR	Odds ratio
PIN	Personal identity number
PMP	Per million people
PPV	Positive predictive value
SRR	Swedish renal register
tTG	Tissue transglutaminase antibodies
VA	Villous atrophy
VTE	Venous thromboembolism

1 INTRODUCTION

Celiac disease (CD) is a common chronic disorder, affecting some 1% of the population in the Western world¹. In individuals with CD, ingestion of the storage proteins of wheat, barley and rye (collectively termed gluten) activate the innate and adaptive immune system. This results in a characteristic inflammation of the small intestine, with atrophy of the small intestinal villi and production of disease-specific antibodies. Intestinal healing is achieved upon treatment with a gluten-free diet (GFD).

Although the expression of human leukocyte antigen (HLA) DQ2 or DQ8 is required for CD pathogenesis², these genotypes are common in the general population³ and do not provide an understanding of why some individuals develop CD and others do not. CD pathogenesis is considered multifactorial, likely dependent on an interplay of genetic and environmental factors such as infant feeding practice⁴ and infections⁵.

The classic presentation of CD includes symptoms of malabsorption such as weight-loss, diarrhea, and growth retardation in children⁶. Although initially considered a largely pediatric gastro-intestinal disorder, the observations that CD may develop at any age, may present with symptoms from other organ systems, or may be asymptomatic has led to a changed view. CD is currently considered a multisystemic disorder that should be considered in all age groups⁶. Individuals with CD suffer increased risk of a number of comorbid conditions, including autoimmune and non-autoimmune disorders such as depression, certain malignancies, and diabetes mellitus type 1¹.

The aim of this thesis was to examine the risk of venous thromboembolism or renal disease in CD, in order to shed further light on the burden of disease in CD and to possibly identify groups at high risk for CD where screening may be warranted. Another aim was to investigate the effect of potential environmental CD risk factors during the first year of life, to improve our understanding of CD pathogenesis and, if possible, suggest future preventive measures.

2 BACKGROUND

2.1 A BRIEF HISTORY OF CELIAC DISEASE

In the second century AD, Aretaus the Cappadocian described a malabsorptive syndrome with chronic diarrhea, dubbing it the “celiac state” after the Greek “koiliakos” (abdomen)⁷. Dr Samuel Gee, an English physician, honored the Greek wording when he, in 1888, published his monograph on the celiac affection; a vivid description of chronic indigestion found in all ages. Gee deduced that “if the patient can be cured at all, it must be by means of diet”⁸. A diet consisting of ripe bananas and rice as the only sources of carbohydrates was advocated to those affected by the syndrome.

Identification of gluten as the dietetic offender was achieved by Dutch physician W.K. Dicke, who noted that children with CD improved dramatically during the Second World War. At this time, staples such as wheat, rye, and barley were scarce. Contraintuitively, a negative effect on the health of these children was seen when famine was relieved and the grains were re-introduced to the diet; an observation leading to the invention of the gluten-free diet⁹.

2.2 DESCRIPTIVE EPIDEMIOLOGY

2.2.1 Prevalence in non-selected populations

Historically CD has been considered a rare disorder. With the evolution of modern diagnostic tools, this view has changed. CD is now generally regarded as a common chronic disorder affecting children and adults worldwide. The prevalence is generally cited as about 1% of the Western population¹⁰, but figures vary according to age, year of measurement and how CD is defined (histopathologic criteria and/or serology)¹¹. An overview of estimates obtained through screening studies in unselected populations is presented in Table 1.

CD occurs worldwide. The highest prevalence reported is among the Saharawi children in Algeria, where serology screening revealed a prevalence of 5.6%²². In Egypt, a more moderate prevalence among children was reported (0.5%)²³. Screening studies in South America showed a high prevalence of CD in Mexico (2.7%), but lower in Brazil and Argentina (0.1%, 0.6% respectively), although the definitions of CD varied (Table 1)^{25,26}. Few screening studies have been performed in Asia. Sood et al reported a 0.3% prevalence of biopsy-verified CD in Indian school-children, however the prevalence was likely underestimated since screening was restricted to children with GI symptoms (Table 1)²⁷.

The prevalence of CD has increased over time. Part of this increase is likely due to an increased use of serological test and the inclusion of cases with only minor mucosal lesions in the presence of positive serology. However, several studies have investigated prevalence in different time periods, suggesting a true increase in prevalence over time that does not depend on changes in clinical practice¹⁷⁻¹⁹. This is coherent with reports of increased prevalence over time in another immune-mediated disease (diabetes mellitus type 1)²⁸.

Table 1. CD prevalence reported by screening studies.

Region and author	Inclusion criteria	Age group	Prevalence
<i>North America</i>			
Fasano (2003) ¹²	tTG and VA or EMA + HLA-DQ2/DQ8	All ages	0.8%
Hill (2000) ¹³	VA	0.5-20 years	1.8%
<i>Europe</i>			
Vilppula (2009) ¹⁴	VA	>55	2.3%
Walker (2010) ¹⁵	VA VA +/- IEL>25/100 and positive serology	Adults	0.7% 1.8%
Myleus (2009) ¹⁶	tTG and VA or IEL>30 + symptoms	12 year olds	2.9%
Lohi (2007) ¹⁷	EMA or previous clinical CD 1978-1980 2000-2001 (two separate samples)	Adults	1.0% 2.0%
Rubio-Tapia (2009) ¹⁸	EMA+tTG positivity 1948-1954 2006-2008 (historical vs modern cohort, matched for age at sampling)	Young adults	0.2% 0.9%
Catassi (2010) ¹⁹	EMA+tTG positivity 1974 1989 (same cohort, repeated measurements)	Adults	0.2% 0.5%
Mustalahti (2010) ²⁰	Previously diagnosed or tTG+EMA positive	Adults	1.0%
<i>Asia/Pacific</i>			
Cook (2000) ²¹	EMA + VA	Adults	1.2%
<i>Africa</i>			
Catassi (1999) ²²	EMA	1.5-14 yrs	5.6%
Abu-Zekry (2008) ²³	Positive serology + biopsy with Marsh stage I-III.	7 months-18 years	0.5%
<i>Latin and South America</i>			
Remes-Troche (2006) ²⁴	tTG	Adults	2.7%
Gandolfi (2000) ²⁵	VA	Adults	0.1%
Gomez (2001) ²⁶	EMA and/or VA	16-79 years	0.6%
<i>Asia</i>			
Sood (2006) ²⁷	VA	3-17 years	0.3%

Abbreviations: EMA, endomysial antibody; IEL, intraepithelial lymphocytes; tTG, tissue transglutaminase antibody; VA, villous atrophy.

2.2.2 Prevalence in selected populations

2.2.2.1 Gender and relatives

As is the case in several other autoimmune disorders²⁹, there is an asymmetric gender distribution of CD prevalence. CD is two to three times more common in women than in men³⁰.

An increased risk of CD has been observed in relatives to individuals with CD. Fasano et al reported increased prevalence among first- and second-degree relatives to individuals with biopsy-verified CD (4.5% and 2.6% respectively) compared with not-at-risk individuals (0.8%), but a positive serology and genetic test sufficed to establish a CD diagnosis in relatives¹². Another study found a CD prevalence of 10% among screened first-degree relatives (small-intestinal biopsy Marsh stage II-III required for CD diagnosis)³¹.

2.2.2.2 Type 1 diabetes mellitus and autoimmune thyroiditis

Individuals with type 1 diabetes mellitus (T1DM) suffer increased risk of CD³², with a reported prevalence of 2-5% in the T1DM population^{12, 32, 33}. Some studies report even higher estimates of CD prevalence in T1DM, for example 12.3% in a Danish study³⁴. In the majority of patients, CD is preceded by T1DM³⁵, however one study from our group found increased risk of future T1DM among individuals with prior CD (prevalence 1.0%, HR, 2.4; 95% CI 1.9-3.0)³⁶. The reasons behind the association between T1DM and CD include shared genetic susceptibility at the human leukocyte antigen (HLA) level³⁷ and in non-HLA regions³⁸, as well as common environmental risk-factors such as infant feeding pattern³⁹. There is no increased risk of diabetes mellitus type 2 among individuals with CD⁴⁰.

Studies have reported an increased prevalence of CD among individuals with autoimmune thyroid disorders^{41, 42}, with one study reporting hazard ratios from 2.0 to 4.0 for future thyroid disease (hyper-, hypothyroidism, and thyroiditis) in individuals with CD⁴³.

2.2.2.3 Turner and Down's syndromes

Increased CD and thyroid autoantibodies have been detected in Turner syndrome⁴⁴. The prevalence of CD among individuals with Turner syndrome has been reported increased (6.4%)⁴⁵. In Down's syndrome, CD prevalence is estimated at 5-19%⁴⁶⁻⁴⁸. CD screening by means of serology is indicated in individuals with Down's syndrome due to the high prevalence of CD in this group, and the often-prolonged patient's delay.

2.2.2.4 Symptoms

Individuals with CD may seek medical advice due to abdominal bloating and pain without signs of malabsorption. This clinical presentation is indistinguishable from that of irritable bowel syndrome (IBS). In a screening study, individuals newly diagnosed with IBS according to the Rome II criteria were at a seven-fold increased risk of having CD, with a CD prevalence of 5% in the IBS population⁴⁹. A later meta-analysis described a four-fold increased risk of CD among individuals with IBS⁵⁰.

Symptoms and signs other than GI symptoms may be indicative of increased risk of CD. In a primary care case-finding study, the most common clinical presentation of CD was anemia, noted in 50% of cases⁵¹. Most studies show a prevalence of biopsy-verified CD of about 5% in individuals with iron-deficiency anemia⁵²⁻⁵⁴. Additionally, low 25-(OH) D-vitamin has been reported to be present in more than 50% of patients with CD⁵⁵.

2.2.2.5 Concluding remarks regarding selected populations

Screening for CD in at-risk populations described above seems reasonable given the elevated risk of CD described in previous studies. Indeed, in children with Down's syndrome, T1DM, family members, and in individuals with autoimmune thyroid disorder, this is common practice⁵⁶. For the other at-risk groups mentioned above, screening by CD serology has been suggested¹¹.

2.2.3 Incidence

Murray et al reported an overall annual incidence of 2.1 per 100,000 person-years based on a study of diagnosed CD in Olmsted county residents from 1950-2001⁵⁷. Interestingly, incidence rates increased over time, starting at 0.9 per 100,000 person-years in 1950-1989, to 3.3 per 100,000 person-years during the 1990's and 9.1 per 100,000 person-years in the last two years of the study. A higher annual incidence of 75 per 100,000 person-years was reported in a Finnish study, based on a screening study of individuals over 55 years of age in 2002-2005¹⁴. Lastly, a Swedish study investigated the incidence of clinically detected CD over time in children under 2 years of age. A rate of 50-60 cases per 100,000 person-years was seen, with a temporary four-fold increase during "the Swedish epidemic" 1985-1987 (see section 2.3.4.1)⁵⁸.

2.3 PATHOGENESIS

2.3.1 Genetics

Genetics play a key role in the development of CD. Studies have found increased prevalence of CD in first- and second degree relatives to individuals with CD^{12, 31}. Additionally, a concordance rate of 75% has been found among monozygotic twins⁵⁹.

CD is considered a multigenetic disorder. The dominant susceptibility locus for CD is the human leukocyte antigen (HLA)⁶⁰, proteins encoded by HLA genes in the major histocompatibility complex on chromosome 6. Individuals with CD are HLA DQA1*05-DQB1*02 (DQ2) and DQA1*03-DQB1*0302 (DQ8) positive⁶¹. However, since about one third of the Western population carry these alleles⁶², and only a fraction of these individuals develop CD, it can be concluded that these genes are required but alone not a sufficient cause of the disorder. HLA-DQ2/DQ8 positivity has been estimated to contribute approximately 40% of the genetic load in CD⁶³.

Recent genome-wide association studies have shed light on non-HLA genes involved in the pathogenesis of CD. It seems there are a great number of different genes involved (to date >100)⁶⁴, each contributing a very small portion of the risk but together constituting 60% of the total genetic load in CD⁶⁵. Many of these genes are immune-related, and have been associated with other autoimmune disorders⁶⁵.

2.3.2 Gluten- sine qua non

Gluten is the collective name given to the storage proteins (prolamines) of wheat, barley and rye. In wheat, these proteins consist of an alcohol-soluble part (gliadin) and a water-soluble part (glutenin). In barley and rye, the toxic counterparts of gliadin are named hordein and secalin, respectively. These proteins confer properties such as viscosity and cohesivity, making gluten an attractive ingredient in the baking of voluminous bread. A common feature of these proteins is that they are rich in glutamine and proline and are poorly digested in the upper gastrointestinal tract by gastric, pancreatic and intestinal brush-border membrane proteases^{66, 67}.

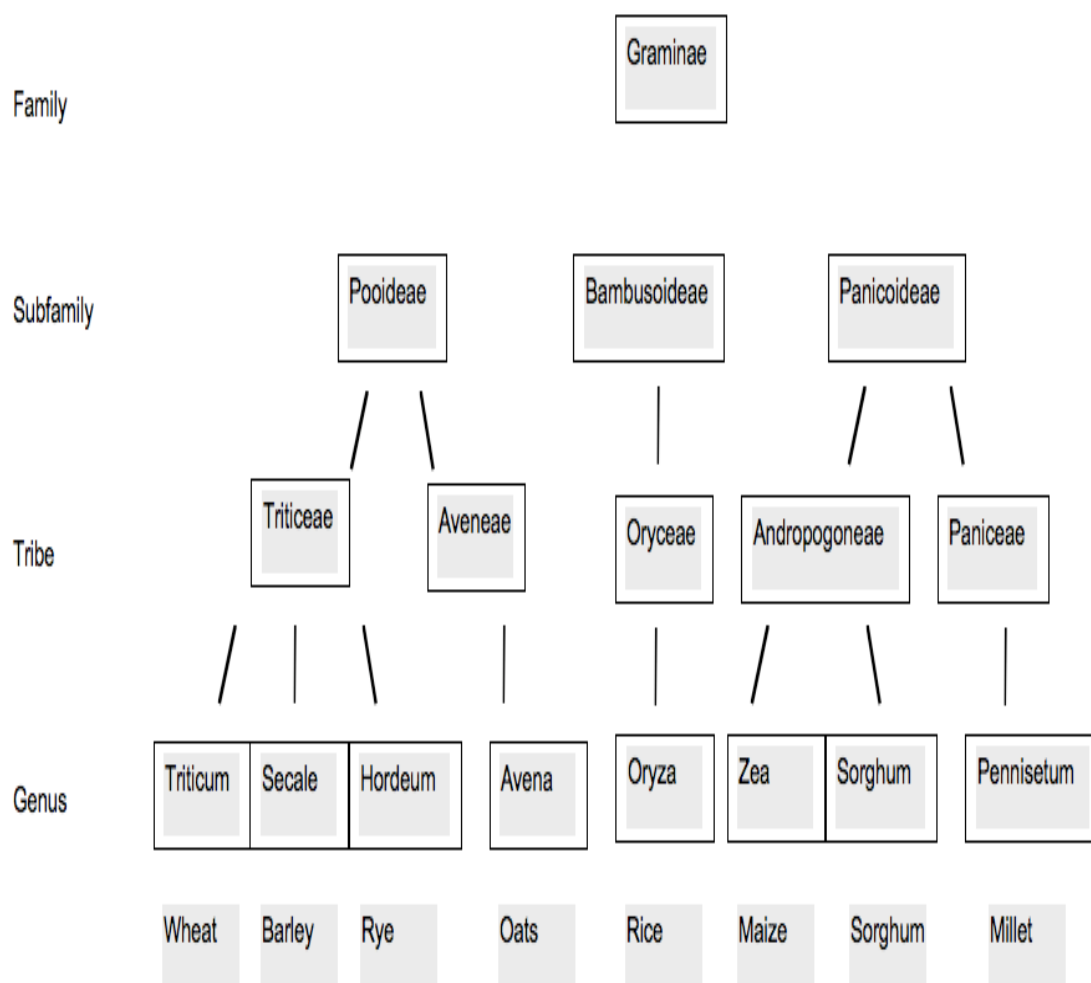


Figure 1. Taxonomy of the grains involved in CD.

As seen in Figure 1, oats are closely related to wheat, barley and rye, belonging to the same subfamily. Avenin, the prolamine in oats, has not been shown to induce CD other than anecdotally^{68, 69}. Oats are generally considered a safe ingredient of a gluten-free diet⁷⁰, although precautions must be made to avoid contamination with gluten. Other grains, such as rice and maize, contain low levels of proline and glutamine and do not confer CD.

2.3.3 Immunological mechanisms

In individuals with CD, incompletely digested gluten peptides cross the intestinal epithelial barrier. The mechanisms allowing a breach of this barricade that is otherwise impermeable to macromolecules remain undisclosed. Increased intestinal permeability due to infections or other stress factors have been suggested to play a role in CD pathogenesis⁷¹. Additionally, there are some evidence suggesting a paracellular pathway due to dysfunctional tight junctions⁷². Furthermore, studies have shown increased transcellular transportation of intact gliadin molecules in individuals with active CD^{73, 74}.

Upon reaching the lamina propria, gluten peptides are deamidated by the enzyme tissue transglutaminase-2 (tTG) (Figure 2). TTG is expressed by many cell types and associates with the extracellular matrix. TTG targets glutamine residuals in extra- and intracellular proteins. It may also covalently link with gluten peptides. When gluten peptides are deamidated, the residual peptide is negatively charged and binds even stronger to HLA-DQ2 (or DQ8) molecules on antigen presenting cells (APCs; macrophages, B-cells and/or dendritic cells). These cells then activate CD4+ T-cells that release inflammatory mediators upon activation. The inflammatory mediators, mainly IFN γ and TNF α , activate matrix metalloproteinases that cause epithelial cell damage and tissue remodelling⁷⁵. Gluten specific T-cells activate B-cells leading to clonal expansion and antibodies against gluten peptides and tTG. Almost all individuals with CD will have developed autoantibodies against tTG⁷⁶.

The adaptive immunity response to gluten peptides in CD has been fairly well described. Interestingly, findings suggest that certain peptide residues, different from the ones that trigger the adaptive immune system, elicit an innate immune response in CD. After binding to epithelial cells or APCs, intra-epithelial lymphocytes (IELs) and natural killer cells are activated by means of IL-15. This leads to epithelial cell killing and increased intestinal permeability. Thus, the adaptive and innate immune responses work in concert to achieve intestinal inflammation, crypt hyperplasia and villous atrophy (VA); the characteristic hallmarks of CD.

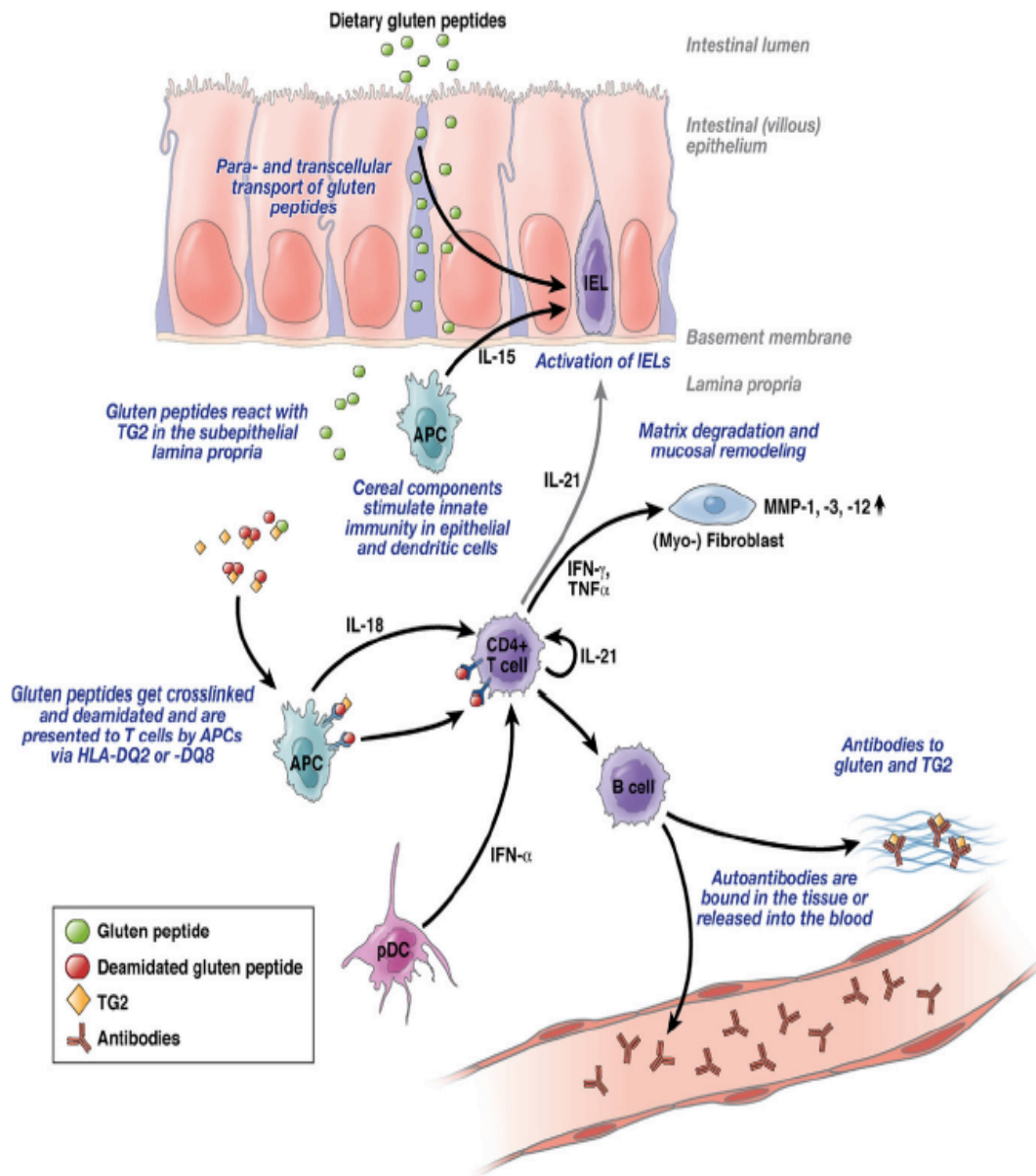


Figure 2. Immunological pathways in CD.

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2.3.4 Environmental factors

Genetics are important in CD, but do not qualify as sufficient causes. Pathologic immunological mechanisms have been elucidated, but are not fully understood. An environmental offender, gluten, has been identified. Yet current research offers no tool of anticipating or preventing the development of CD in patients. It is increasingly clear that CD is a multifactorial disease, in which the interplay of genetic and environmental factors other than gluten contributes to disease occurrence. In Finland, screening studies showed a 1.5% prevalence of CD autoantibodies among children age 7-16 years⁷⁷, but 2% among screened adults¹⁷. This indicates increased CD development over time in susceptible individuals, and further denotes the importance of environmental risk factors (or protective factors) in CD pathogenesis.

2.3.4.1 Infant feeding practice

The first two years of life represent a challenging period for children's nutrition and health. The velocity of growth is rapid, and the metabolic rate is high. In addition, the infant's immune system and gastrointestinal function is immature, limiting possible food sources and rendering the infant sensitive for food-borne infections⁷⁸.

The current WHO guidelines on breastfeeding recommend exclusive breastfeeding during the child's first six months of life⁷⁹. Exclusive breastfeeding is defined as the consumption of no other food or liquids except breast milk and small amounts of medicines or vitamin-mineral supplements. At 6 months of age, complementary feeding should be introduced in order to fulfill the child's energy and nutrition requirements. It is recommended that breastfeeding be continued until two years of age, or beyond⁸⁰. Guidelines offered by the Swedish Board of Health and Welfare (Socialstyrelsen) and the National Food Agency (Livsmedelsverket) regarding breastfeeding duration have been modified to comply with WHO standards (Figure 3).

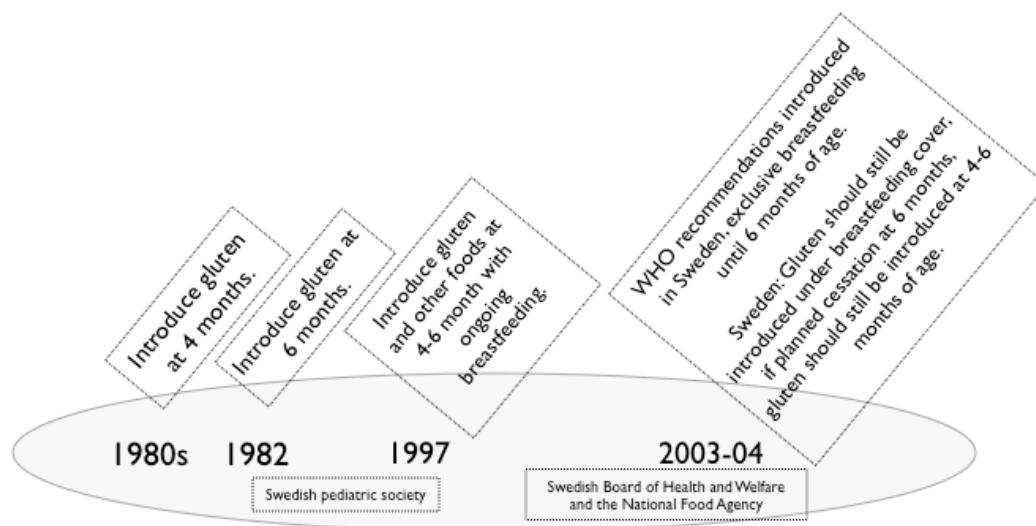


Figure 3. Infant feeding recommendations in Sweden over time

In 1985-1987 the incidence of CD in children 0-2 years of age increased dramatically, from 50-60 cases per 100,000 person-years to 200-240 cases per 100,000 person-years (see Figure 4)⁵⁸. This incidence remained elevated until 1995, when the incidence rate dropped to the same level as before the so-called “Swedish epidemic”. The start of the epidemic coincided with increased consumption of gluten containing cereals in 1981-1983 and recommendations from the Swedish pediatric society to postpone gluten introduction until 6 months of age (see Figure 3). The end of the epidemic, in 1995, concurred with an increased number of children being breastfed at time of gluten introduction (from 54% to 76%), reduced consumption of gluten flour in the first year of life, and changed recommendations regarding breastfeeding suggesting that parents introduce gluten from 4 months of age whilst continuing breastfeeding⁵⁸. In all, the “Swedish epidemic” supports the hypothesis that infant feeding practice affects the risk of CD in children.

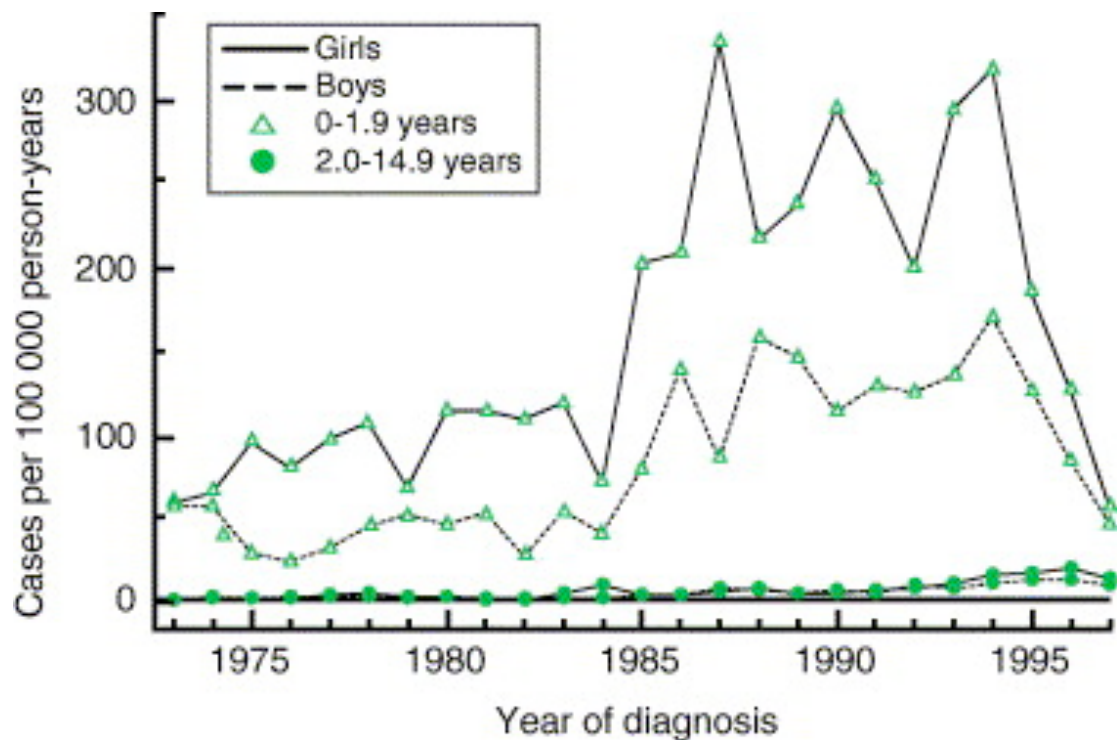


Figure 4. Incidence of CD in Swedish children aged 0-2 years over time.

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Michaelsen et al compared the intake of wheat at 9 and 12 months of age in Danish compared with Swedish infants and found that Swedish infants had a substantially larger wheat intake⁸¹. Interestingly, Danish CD incidence rates have been reported lower than those of other European countries⁸². One study found higher incidence of CD in Swedish compared with Danish children with comparable breastfeeding patterns but earlier gluten introduction in the Danish group⁸³. Interestingly, Swedish children presented with CD symptoms at a significantly lower age (mean 1.5 vs 5.5 yrs, $p < 0.01$). It has been suggested that early (< 3 months of age³⁹ or < 2 months of age⁸⁴) or late (> 7 months of age)³⁹ introduction of breastfeeding increase the risk of CD. However, Ivarsson et al found that a greater number of healthy individuals had a late (after 7-12 months) gluten introduction when compared to individuals with CD⁸⁵.

Several studies have investigated the effect of breastfeeding on the risk of CD. Although most studies suggest a greater risk for CD in infants with shorter breastfeeding duration⁸⁴⁻⁸⁸, research findings are inconsistent⁸⁹⁻⁹¹. The majority of these studies were retrospective. Furthermore, it is unclear whether a shorter duration of breastfeeding reduces the risk of CD or merely delays disease onset. It has also been debated if the postulated effect of breastfeeding on CD may be best understood by later gluten introduction upon weaning in these babies. A recent meta-analysis including 6 studies⁸⁴⁻⁸⁹ showed that breastfeeding at time of gluten introduction may be protective of future CD (pooled odds ratio (OR) 0.48, 95% CI 0.40-0.59) (see Figure 5). In conclusion, the body of previous research suggests that infant feeding practice is a factor that affects CD pathogenesis.

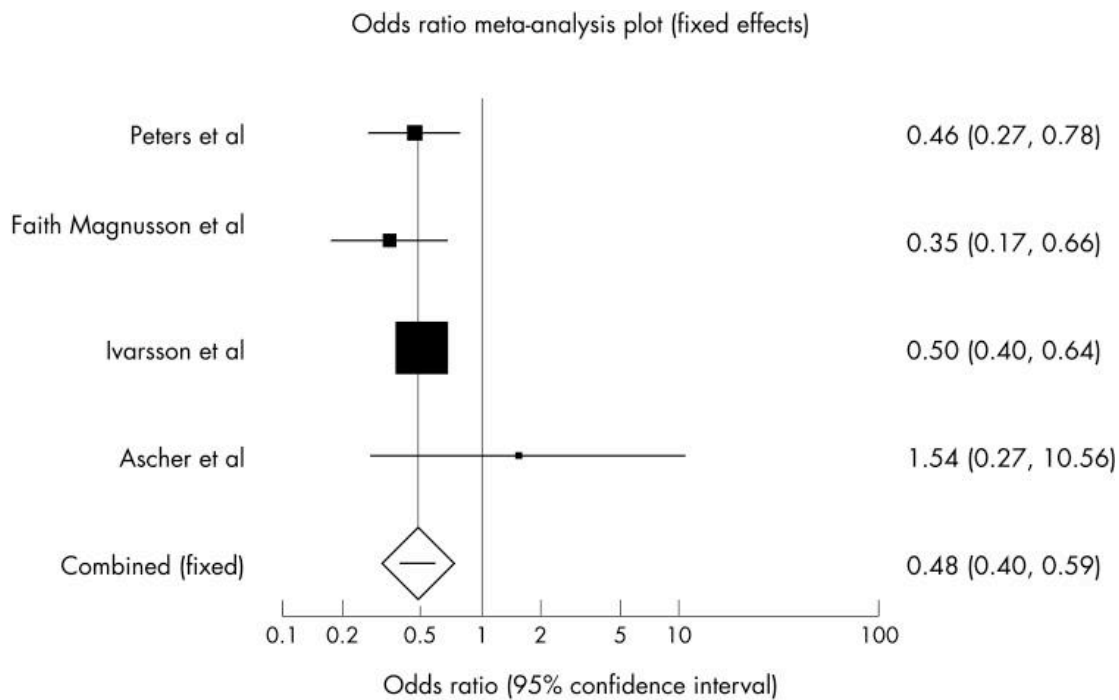


Figure 5. Odds ratios (95% CI) of effect of breast-feeding at the time of gluten introduction on development of CD.

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2.3.4.2 Microflora and perinatal factors

It has been hypothesized that an altered bacterial intestinal flora might impact the risk of CD. Indeed, individuals with CD seem to have different metabolic characteristics of their microflora compared to healthy controls⁹². Interestingly, these differences in fecal microflora composition were evident in treated as well as in untreated celiacs⁹², and in screening detected cases⁹³. Previous studies have suggested increased risk of CD in individuals delivered by elective caesarean delivery⁹¹. A recent case-control study found increased risk of CD in elective caesarean deliveries (adjusted OR, 1.15; 95% CI 1.04 – 1.26) but not in emergency caesarean deliveries⁹⁴. In elective caesarean deliveries, all children have avoided contact with the maternal vaginal bacterial flora. The positive association with elective caesarean delivery thus supports the hypothesis that an altered gut microflora confers an increased risk of CD.

Maternal smoking during pregnancy is reported to confer a small risk increase of CD in offspring (OR, 1.10; 95% CI 1.01-1.19)⁹⁵, however, another study reported a higher estimate (Relative risk, 2.12; 95% CI 1.19–3.79)⁹⁰. Being small for gestational age has been shown to increase the risk of future CD (OR, 1.45; 95% CI 1.20-1.75)⁹⁵. Neonatal infections, prospectively reported in a register study, conferred an increased risk of future CD (OR, 1.52, 95% CI 1.19–1.95)⁹⁵.

2.3.4.3 Infectious disease

Seasonal variance in incidence is a common characteristic of infectious diseases. It's therefore interesting that individuals born in the summer suffer increased risk of CD (RR, 1.4; 95% CI 1.2- 1.7)⁹⁶. Could infectious disease be an environmental factor of

interest in CD pathogenesis? Indeed, Stene et al found increased risk of CD autoimmunity in children with higher rotavirus infection frequency (rate ratio for trend per increase in number of infections, 1.94; 95% CI 1.04–3.61, $p = 0.037$)⁹⁷. Additionally, Decker et al found a nearly threefold risk of postnatal GI disorders, including GI infections, in children with CD. No associations with other infections were found⁹¹. Lastly, Kagnoff et al reported increased levels of anti-adenovirus antibodies in individuals with CD⁹⁸. Since a particular adenovirus protein share amino acid sequence homology with gliadin, the authors hypothesized that perhaps certain antigens can confer an increased CD risk by immunological cross reactivity. Another potential mechanism whereby infectious disease could affect CD pathogenesis is by increased intestinal permeability.

In conclusion, there are a number of environmental factors suggested to play a role in CD pathogenesis, including infant feeding practice and infectious disease. Many questions, however, remain unresolved. Is breastfeeding really protective of CD or are current studies merely reporting a symptom delay or recall bias? Or is the true offender a high gluten load during infancy? Furthermore, what is the role of infectious disease in CD pathogenesis? Under what circumstances, if any, can an infection trigger CD, and which are the relevant pathogens?

2.4 CLINICAL PRESENTATION

CD was initially described as a syndrome of steatorrhea, diarrhea, and malabsorption in children⁹⁹. A classic mode with GI symptoms as the dominating symptoms at presentation has become less common over time in children and adults^{100, 101}. Overall, there has been a shift towards milder symptoms, likely due to increased use of CD serology. Before 1993, 73% of adults with CD presented with diarrhea. This symptom was less common after 1993 (43%) when serologic testing was introduced as a diagnostic tool¹⁰². At the Celiac Disease Center at Columbia University, New York, the most common modes of presentation in adults were diarrhea (40%), anemia (15%), screening (10%), bone disease (6%) and incidental findings at upper endoscopy (6%). In children, the most common findings were growth issues (26%), screening (23%), abdominal pain (22%) and diarrhea (9%)¹¹. Weight loss is an uncommon symptom of CD, and it is of clinical importance to note that CD may occur also in overweight individuals¹⁰³.

The spectrum of clinical presentations in modern CD is wide, from asymptomatic CD detected upon screening in at-risk populations, to disease with extra-intestinal manifestations (atypical disease), and, lastly, “classic” CD. Although CD is defined as a lesion of the small-intestinal mucosa, the following symptoms and disorders may suggest CD and warrant investigation: ataxia, anemia⁵², neuropathy, depression, osteoporosis, chronic thrombocytopenic purpura, Addison’s disease, and tuberculosis¹¹.

In conclusion, there has been a shift in the presentation of CD over time. This shift may be explained by changed disease characteristics; however, a more likely explanation is a coinciding development of diagnostic tools¹⁰⁴.

2.5 DIAGNOSING CELIAC DISEASE

2.5.1 Diagnostic criteria

Current diagnostic criteria for CD are based on guidelines issued by the European Society for Pediatric Gastroenterology and Nutrition (ESPGHAN)¹⁰⁵ and have been extrapolated to adults¹⁰⁶⁻¹⁰⁸. For a CD diagnosis, characteristic small intestinal mucosal abnormalities are required, as well as clear-cut clinical remission on a strict gluten-free diet. A positive CD serology that reverts to normal after dietary treatment strengthens the suspicion of CD, but is by itself not enough to establish the diagnosis. Small intestinal biopsy remains the gold standard in diagnosing CD. In asymptomatic patients and in cases with a weak clinical response upon initiation of dietary treatment a control biopsy is required to verify histological remission on a gluten-free diet. A biopsy after gluten provocation may be useful in individuals with unclear diagnosis, and should be considered in children less than 2 years of age¹⁰⁹.

A recent consensus paper suggested definitions of CD and related disorders (see Table 2)¹¹⁰.

Table 2. The Oslo definitions¹¹⁰ of CD and related disorders.

CD	A chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals.
Asymptomatic CD	CD not accompanied by symptoms even in response to direct questioning at initial diagnosis.
Classical CD	CD presenting with signs and symptoms of malabsorption. Diarrhoea, steatorrhoea, weight loss or growth failure is required.
Non classical CD	CD presenting without signs and symptoms of malabsorption.
Subclinical CD	CD that is below the threshold of clinical detection.
Potential CD	Individuals with a normal small intestinal mucosa who are at increased risk of developing CD as indicated by positive CD serology.
CD autoimmunity	Increased tTG or EMA on at least two occasions when status of the biopsy is not known. If the biopsy is positive, then this is CD, if the biopsy is negative then this is potential CD.

2.5.2 The use of serological tests

Serological testing has since the 1990's developed into an important tool in determining which individuals should undergo small intestinal biopsy. This practice has led to an increased understanding of the CD spectrum. Furthermore, it has contributed to an understanding that CD is a more common disorder than previously thought.

Antibodies against gliadin (AGA), endomysium antibodies (EMA) and tissue transglutaminase antibodies (tTG) are commonly considered. The use of AGA (IgG and IgA) is limited, due to a comparatively low sensitivity and specificity (see Table 3)⁷⁶. In children < 18 months of age, AGA in combination with tTG is preferred since many children lack EMA and tTG¹¹¹. In adults and children > 18 months, EMA or tTG are recommended whereas AGA is not. A recently developed assay for antibodies against deamidated gluten peptides seems promising and may be used to increase diagnostic accuracy in children¹¹².

Table 3. Performance of serologic screening tools in CD⁷⁶.

	Sensitivity (95% CI)	Specificity (95% CI)	Prevalence
IgA AGA adult	0.75-0.90 H	0.80-0.90 H	36%
IgA AGA children	0.80-0.95 H	0.80-0.95 H	36%
IgA EMA ME adult	0.974 (0.957-0.985)	0.996 (0.988-0.999)	40%
IgA EMA ME children	0.961 (0.945-0.973)	0.974 (0.963-0.982)	40%
IgA tTG HR adult	0.981 (0.901-0.997)	0.981 (0.958-0.991)	40%
IgA tTG HR children	0.957 (0.903-0.981)	0.990 (0.946-0.998)	40%

HR, human recombinant; ME, monkey esophagus.

H= significant heterogeneity according to Pearson's chi square test.

The endomysium is a connective tissue protein found in collagenous matrix surrounding smooth muscle cells. The EMA test uses either monkey esophagus or human umbilical cord as substrate. The occurrence of antibodies is measured with an immunofluorescent staining technique that requires manual evaluation¹⁰⁷. In 1997, Dietrich et al identified tTG as the antigen in EMA¹¹³, leading to the development of ELISA methods for tTG. Nowadays, most laboratories use human recombinant tTG as substrate. Even though the EMA outperforms the tTG in terms of specificity, tTG has important advantages being less expensive and labour intensive, quantitative instead of operator dependent, quicker and not using primate tissue. In conclusion, most studies report sensitivities and specificities for tTG and EMA > 95%, and both tests are considered useful in CD diagnostics¹¹⁴.

IgA deficiency is a state where an individual has decreased levels of IgA but normal levels of serum IgG and IgM. In selective IgA deficiency, no IgA at all can be detected. Selective IgA deficiency is ten times more common in individuals with CD¹¹⁵, therefore total IgA needs to be assessed when performing serological tests in suspected CD. If selective IgA deficiency cannot be ruled out, IgG EMA and IgG tTG tests can

be used⁵⁶. Since these have lower specificity and sensitivity¹¹⁶ than IgA based tests, biopsy may be considered independently of IgG serology results.

Henceforward, the term “CD serology” is defined as a term that includes endomysium-, transglutaminase-, or deamidated gliadin antibodies, and in small children also gliadin antibodies for the assessment of CD¹¹⁰.

CD serology has a high specificity for CD⁷⁶ and a high negative predictive value (NPV). A high NPV makes CD serology particularly useful in ruling out the disease, stating that the likelihood of not having the disease is high if the test is negative. In contrast, the positive predictive value (PPV) of CD serology is dependent on the prevalence of the disorder. Consider a sensitivity of 99%. One case in 100 cases tested will have a false-positive test result. Given a CD prevalence of 1%⁵⁶, the test will yield 2 positive results of which 1 is negative, and the PPV will be 50%, i.e. the probability of having CD given a positive CD serology is low. In studies by Hopper et al¹¹⁷ and Hadithi et al¹¹⁸, the NPV of tTG screening was >99% with reported PPV at 28.6% and 73% respectively in populations with a 3.9% and 3.46% CD prevalence. The low PPV is an important feature of CD serology that supports the continued use of small intestinal biopsy before prescribing a life-long dietary treatment. Another important aspect is the fact that seronegative CD does occur¹¹⁷, thus biopsy is recommended irrespective of negative CD serology in the light of high CD suspicion¹⁰⁵. The increased availability of over-the-counter rapid antibody tests¹¹⁹ may lead to individuals initiating a gluten-free diet without prior biopsy.

2.5.3 Genetic testing

Since virtually all individuals with CD are HLA-DQ2 or DQ8 positive⁶¹, a negative HLA test is forceful in ruling out the disease, i.e. the NPV is very high. However, since these HLA types will also be found in about 1/3 of the general population⁶², a positive result will merely confirm the possibility of CD. A positive test result is not in itself suggestive of CD, since only 3% of individuals carrying these alleles will develop CD. A HLA test may be particularly useful in ruling out CD in IgA deficient individuals or individuals with high heredity for CD, and in individuals on a GFD who have not undergone biopsy,

2.5.4 Small intestinal biopsy

Small intestinal biopsy is the gold standard of diagnosing CD⁵⁶. The indication for small intestinal endoscopy and biopsy is often a positive CD serology. In a Swedish study, 100% of pediatricians and 96% of gastroenterologists reported that they perform small intestinal biopsy in at least 9/10 patients prior to establishing a CD diagnosis¹²⁰.

The mucosal changes seen in CD, originally classified by Marsh¹²¹, is a spectrum ranging from near-normal mucosa to intestinal inflammation and VA. Marsh stage I is characterized by increased counts of intraepithelial lymphocytes (IELs) (>30 IELs per 100 epithelial cells), and stage two of raised IELs in conjunction with crypt hyperplasia (Figure 6). Marsh I and II are considered early changes in individuals predisposed to CD, however these abnormalities may be caused by GI disorders other than CD, such as giardiasis, helicobacter pylori gastritis and viral gastroenteritis (see Table 5)¹²². Marsh stage III is divided in 3 groups; Marsh type IIIa (partial VA), Marsh type IIIb (subtotal VA) and Marsh type IIIc (total VA) (Figure 6). An overview of small

intestinal histopathological classifications is offered in Table 4. Swedish intestinal biopsies are classified according to the SnoMed system, that is based on the same criteria as the Marsh classification for duodenal and jejunal biopsies.

Table 4. Overview of small intestinal histopathology classifications in CD.

Classification	Villous atrophy (VA)		
	Stage IIIa	Stage IIIb	Stage IIIc
Marsh classification			
Marsh description	Flat, destructive		
SnoMed codes	M58, D6218, M58005	M58, D6218, M58006	M58, D6218, M58007
KVAST/Alexander classification	III Partial VA	IV Subtotal VA	IV Total VA

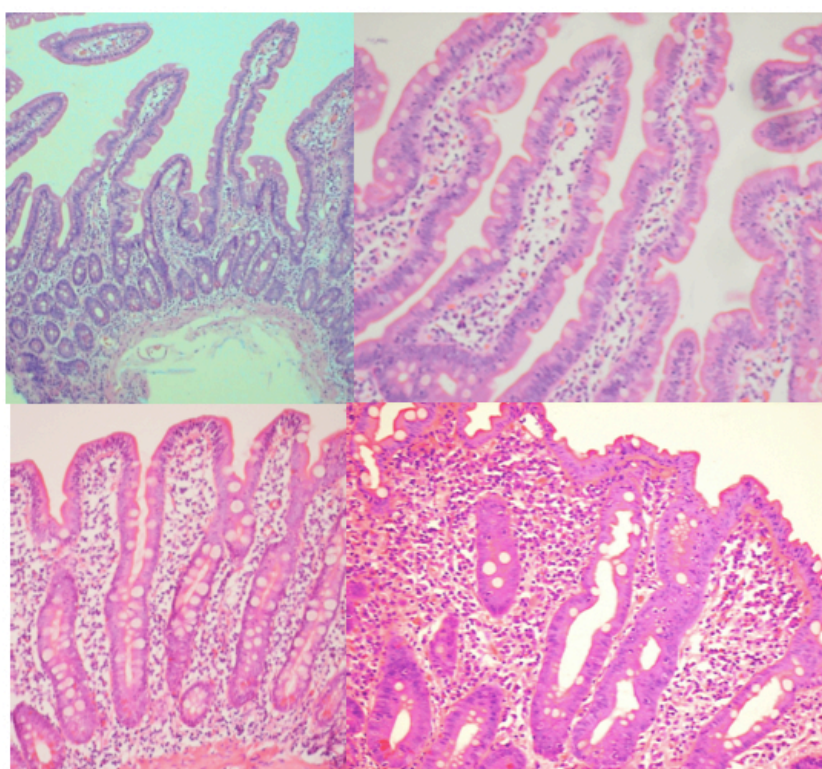


Figure 6. Marsh stages 0 (normal, upper left), I (upper right), II (lower left) and III (lower right). Acknowledgement, dr Marjorie Walker, Histopathology, Faculty of Medicine, Imperial College London

The celiac lesion may be patchy, therefore it is recommended that 4-6 biopsies are acquired from the proximal small intestine⁵⁶, including one biopsy specimen from the duodenal bulb¹²³, to increase the likelihood that VA is detected should it be present. In

CD, macroscopic aberrations might be present such as mucosal scalloping, an absence of folds, and a mosaic pattern of the mucosa between the folds. However, since only total and subtotal VA may be visible macroscopically, this visualization should not replace biopsy. Biopsy specimens should be of sufficient size, carefully oriented and mounted with the villous side up, allowing for cross sectioning rather than tangential sectioning which might be misleading.

All that is flat is not CD, but most often VA is CD. In a Swedish data-set, 95% (108/114) of patients with VA had CD, indicating that the specificity of CD in VA is high¹²⁰. Internationally, other causes of VA may be more common (Table 5)¹²².

Table 5. Differential diagnoses to consider in CD investigation¹²².

Increased intraepithelial lymphocytes
<ul style="list-style-type: none"> - Allergies to proteins other than gluten (eg, chicken, cow's milk, eggs, fish, rice and soy; entities cause both raised intraepithelial counts and villous architectural changes) - Autoimmune conditions, various (eg, systemic lupus erythematosus) - Bacterial overgrowth - Blind loop syndrome - Dermatitis herpetiformis - Giardiasis - Graft-versus-host disease - Helicobacter pylori - Inflammatory bowel disease - Irritable bowel syndrome - Microscopic colitis - Non-steroidal anti-inflammatory drugs - Tropical sprue (entities cause both raised intraepithelial counts and villous architectural changes) - Viral enteritis
Crypt hyperplasia or villous flattening
<ul style="list-style-type: none"> - Allergies to proteins other than gluten (eg, chicken, cow's milk, eggs, fish and soy; entities cause both raised intraepithelial counts and villous architectural changes) - Autoimmune enteropathy - Collagenous sprue - Common variable immunodeficiency - Drug-induced - Hypogammaglobulinaemic sprue - Ischaemia - Kwashiorkor - Radiation therapy - T cell lymphoma, associated enteropathy - Zollinger-Ellison syndrome

2.5.5 To screen or not to screen?

The WHO has established criteria for mass screening (Table 6)¹²⁴. CD fulfils several of these criteria; about 2/3 of individuals with CD are asymptomatic¹²⁵, it is a common disorder with a prevalence of about 1% worldwide⁵⁶, and there is an available treatment (GFD). Yet screening in CD is debatable, for several reasons. The low PPV of celiac serology^{117, 118} would result in a large number of false-positive tests, which may cause harm in terms of anxiety and unnecessary small-intestinal biopsies. It would also lead to a large increase in health-care workload and be costly. One can question the availability of a treatment; adherence to a GFD is expected to be low in screening detected cases. Furthermore, current research has not reached a consensus regarding the benefits of a GFD in individuals with asymptomatic disease, and we cannot rule out a complete lack of benefits provided by a GFD in asymptomatic CD. Canavan et al found no increased mortality in individuals with undiagnosed CD¹²⁶, however, another study found a four-fold increased risk of death in individuals with undiagnosed CD¹⁸. Furthermore, Corrao et al found increased mortality in individuals with CD and signs of malabsorption (standardized mortality rate (SMR), 2.5; 95% CI 1.8-3.4) but not in those diagnosed because of minor symptoms¹²⁷.

Do interventions reduce the risk of complications in asymptomatic CD? How should we treat individuals with potential CD (positive CD serology but normal mucosa)? Will these individuals benefit from a GFD? These questions will likely require answers before national screening can be recommended. However, there is some support for screening or active case finding in high risk groups¹²⁸. Screening in high risk groups and in individuals with symptoms is a feasible and cost-efficient strategy that has the additional advantage of raising awareness regarding CD¹²⁹.

Table 6. The WHO criteria¹²⁴ for mass screening.

1. The condition should be an important health problem.
2. There should be a treatment for the condition.
3. Facilities for diagnosis and treatment should be available.
4. There should be a latent stage of the disease.
5. There should be a test or examination for the condition.
6. The test should be acceptable to the population.
7. The natural history of the disease should be adequately understood.
8. There should be an agreed policy on whom to treat.
9. The total cost of finding a case should be economically balanced in relation to medical expenditure as a whole.
10. Case-finding should be a continuous process, not just a "once and for all" project.

2.6 TREATMENT

2.6.1 The gluten-free diet

When the CD diagnosis is established, a life-long gluten-free diet (GFD) is prescribed. This is currently the only available treatment for CD. The GFD is devoid of wheat, barley and rye. Symptom reduction is usually reported after days or weeks after initiation of treatment⁵⁶, however mucosal abnormalities may persist. Lee et al noted

that only 21% of individuals on a GFD had normal mucosa after an average of 8.5 years of treatment¹³⁰.

The acceptance of wheat starch and oats in the diet of individuals with CD has been debated but is currently accepted in many European countries including Sweden. Available studies suggest that oats may be safely introduced in the diet of adults^{131, 132} and children^{70, 133} with CD. In many regions, however, the inclusion of oats is not consistently recommended due to high levels of cross contamination with gluten-containing grains during growing, transportation and milling⁵⁶. Wheat-starch derived gluten-free products contain trace amounts of gluten. A randomized study comparing individuals including these products in their diet with individuals on a natural GFD found no differences in morphological and clinical response¹³⁴.

Even though an ideal diet for an individual on CD is strictly gluten-free, this is not realistic given gluten contamination of gluten-free products¹³⁵. It has been calculated that the average GFD contains 20-100 parts per million (ppm) gluten/day. The “safe threshold” of gluten intake has been debated. Catassi et al suggested that daily gluten intake should rest below 50 mg/day, based on a prospective double-blind placebo controlled trial with histologic examination¹³⁶. Given a daily gluten-free flour consumption of 80 g, Collin et al suggested an accepted daily intake of 100 ppm (30 mg)¹³⁵. The WHO Codex Alimentarius updated its guidelines in 2008, stating that naturally gluten-free foods can contain up to 20 mg gluten/kg (20 ppm) and 100 mg gluten/kg in wheat starch-derived gluten-free products¹³⁷. These recommendations obviously set out to prevent mucosal damage in CD patients and they reflect current knowledge regarding gluten tolerance in CD. However, another important aspect is the availability and cost of gluten-free products. Should the diet be too awkward and expensive, adherence rates would likely decrease. According to the Swedish Consumer Agency, CD infers increased yearly food costs of about 2760 SEK (€290) for children, 3890 SEK (€410) for women and 4715 SEK (€500) for men¹³⁸. A few counties in Sweden currently provide subsidies to individuals with CD.

At diagnosis, vitamin status should be assessed by measuring the serum folate, vitamin B12, and vitamin D (25-hydroxy) in patients with CD. Deficiencies should be substituted. The GFD and gluten-free products are often deficient of fiber, iron, calcium, vitamin D, magnesium, folate, niacin, B12 and riboflavin¹³⁹. Few gluten-free products are enriched, and vitamin B deficiency may develop even after ten years of adherence to a GFD¹⁴⁰. Therefore, all patients are advised to take a gluten-free multivitamin¹¹.

Although the GFD accomplishes symptom relief in most patients and mucosal improvement, albeit imperfect, in some, the treatment is challenging. There are currently several ongoing research projects investigating the possibility of nondietary treatments in CD, for which there exists an unmet need. Interesting substances in pipeline include oral proteases for gluten detoxification (phase I+II), immunomodulatory substances including monoclonal antibodies, and a peptide vaccination (phase I)¹⁴¹. In the longer perspective, increased understanding of environmental factors in the development of CD could enable primary prevention strategies.

2.6.2 Benefits of a gluten-free diet

In individuals with symptomatic CD, the most obvious benefit of the GFD is a swift clinical improvement characterized by subjective and objective symptom relief. Studies show that treatment with GFD improves bone mineralization in children¹⁴² and adults¹⁴³, reverses anemia and iron deficiency¹⁴⁴, and restores body composition in children¹⁴⁵. In individuals with gastro-intestinal symptoms (classic CD), quality of life is improved after initiating a GFD¹⁴⁶. No consensus has been reached regarding the effect of GFD on quality of life in individuals with asymptomatic CD¹⁴⁶⁻¹⁴⁸.

Upon initiation of a GFD, clinical, serological and histological improvement should follow. Mucosal recovery is not instant. Lee et al reported that only 21% of individuals on a GFD had normal mucosa after an average of 8.5 years of treatment¹³⁰. Similarly, Hopper et al noted that one third (16/48) of patients on a GFD for one year had remaining VA at follow-up biopsy¹⁴⁹. It was noted that 44% (7/16) of individuals with VA had seroconverted after one year of GFD, suggesting that tTG antibody titres correlate poorly with mucosal lesion severity¹⁴⁹.

It has been suggested that adherence to a GFD reduces mortality¹²⁷, risk of autoimmune disorders¹⁵⁰ and lymphoma in CD¹⁵¹. Ventura et al reported increased risk of autoimmune disorders in individuals with CD diagnosed after 10 years of age (23.6%), compared with individuals diagnosed before 2 years of age (5.1%, $p < 0.001$)¹⁵⁰, suggesting that duration of exposure to gluten may affect the risk of complications in CD. Adherence to a GFD has been associated with decreased mortality rate¹²⁷, however, a prospective cohort study reported decreasing mortality ratios with increasing age at CD diagnosis¹⁵². It is important to note that a direct causality of gluten exposure duration on the risk of comorbidity and mortality and CD has not been formally tested. Studies merely imply an association without disentangling *duration of gluten exposure* from *age at CD diagnosis*. Furthermore, few studies include enough follow-up time to assess the risk of outcomes common in adulthood, such as cancer, in children diagnosed with CD.

In conclusion, individuals with clinically detected CD seem to benefit from the GFD in terms of symptom relief and reversal of clinical abnormalities, as well as reduced risk of future complications. Research available to date is non-conclusive when it comes to benefits of the GFD in individuals with screening detected CD.

2.6.3 Achieving and monitoring adherence

The GFD is a complicated treatment that requires an active and well-informed patient. To optimize adherence, newly diagnosed patients should receive information and medical nutritional education from skilled dietitians. There are patient support groups in many regions, contributing valuable forums. The patient with CD should be evaluated at regular intervals by a multi-professional health care team including a physician and a dietitian. Follow-up visits should include assessment of adherence, physical examination, assessment of symptoms as well as growth, particularly in children. At these visits, the GFD should be promoted. The responsible physician should be aware of common complications in CD such as osteoporosis, vitamin deficiencies and other autoimmune disorders, and test for these when symptoms appear.

There is no optimal method to monitor dietary adherence. Although EMA positivity did not predict mucosal recovery, it correlated with reported poor dietary adherence in 80%

of cases¹⁵³. EMA or tTG positivity after 1 year of treatment is indicative of poor dietary adherence¹⁵⁴. Although CD serology might be helpful, assessment of dietary adherence by trained interviewers is recommended, since this method is non-invasive, inexpensive, and correlates with mucosal recovery¹⁵⁵.

In the care of individuals with CD, it may be useful to consider varying adherence rates. The level of adherence achieved has been reported lower in individuals with screening detected CD compared with individuals diagnosed due to symptoms¹⁵⁶, although a Finnish study found no difference between the groups¹⁵⁷. A Swedish study reported higher adherence rates in individuals diagnosed at an age of < 4 years of age compared with individuals diagnosed later in life (80% vs. 36%)¹⁵⁸.

2.6.4 Non-responsive CD

It is common that individuals with CD respond poorly to the GFD. In a study by Leffler et al, non-responsive CD was defined as a failure to respond to a GFD of at least 6 months, or the re-occurrence of symptoms or laboratory abnormalities typical of CD whilst on a GFD¹⁵⁵. 10-19% of individuals with CD fulfilled these criteria. The most common reasons for the unresponsiveness were dietary transgressions (36%), IBS (22%) and refractory CD (RCD) (10%).

When unresponsive CD is discovered, further investigations should include; 1) re-evaluation of the initial CD diagnosis including exclusion of other causes of VA; 2) assessment of dietary compliance and; 3) CD serology that might indicate dietary transgressions⁵⁶. If poor adherence is detected, contact with a dietician, referral to a support group and regular follow up is warranted. Non-responsive CD in individuals with strict adherence warrants further investigations to exclude the occurrence of intestinal malignancies or refractory CD.

2.7 ASSOCIATED DISORDERS

Individuals with CD suffer increased risk of a number of associated disorders and complications, such as malignancies, death, liver disease, anemia, thyroid disease and depression. Some associations, such as osteoporosis and anemia, are caused by nutritional deficiencies, some by shared genetics. The mechanisms underlying other associations remain poorly understood. Some complications of relevance are discussed below.

2.7.1 Mortality in CD

With few exceptions¹⁵⁹, research has shown an increased risk of death in CD. A recent Swedish prospective population-based cohort study showed increased risk of death in CD (HR, 1.39; 95% CI 1.33-1.45)¹⁵². This estimate is lower than those reported by previous studies^{160, 161}, likely due to the increased use of antibody screening rendering cases diagnosed at a lower disease activity. Furthermore, most previous studies were based on inpatients or few clinical units, presenting a selection bias favoring the inclusion of individuals with complicated disease. Significantly increased mortality ratios due to cardiovascular disease, respiratory disease, and malignancies were reported¹⁵².

2.7.2 Dermatitis herpetiformis

Dermatitis herpetiformis is a cutaneous manifestation of CD. It is a blistering skin condition usually located on knees, elbows, buttocks and scalp. Diagnosis is made after skin biopsy. Most individuals with dermatitis herpetiformis have signs of mucosal inflammation and/or VA. The rash responds to a GFD, however in a delayed fashion. Treatment with dapsone is usually administered to relieve the rash during GFD initiation¹⁶².

2.7.3 Refractory CD

Refractory CD (RCD) is defined by the presence of persistent or recurrent malabsorptive symptoms (e.g. diarrhea, abdominal pain or weight loss), and VA despite strict adherence to a GFD for at least 12 months, in the absence of other causes of non-responsive CD and malignancy and after confirmation of the initial CD diagnosis¹⁶³. RCD is a diagnosis of exclusion. In RCD, patients may never have responded to treatment or relapsed despite adherence and initial response. CD serology is most often negative, however a positive test does not rule out RCD. In establishing RCD, a careful assessment of GFD adherence must be performed. In RCD type I, IELs have a normal phenotype whereas RCD type II is characterized by an aberrant IEL population⁵⁶. The existence of a clonal aberrant T-cell population is prognostic; in individuals with RCD II mortality is high (five-year mortality rate 40-58%) mainly due to an increased risk of enteropathy-associated T-cell lymphoma (EATL). The prognosis of RCD type I is much better¹⁶⁴, although the rate of complications and mortality seems to be significantly higher than in responsive CD¹⁶⁵.

Treatment of RCD includes nutritional support and repletion of vitamins and minerals, as well as support to achieve a strict GFD. Corticosteroids have induced improvement in most cases, immunosuppressive drugs may be beneficiary, and autologous hematopoietic stem-cell transplantation have been successful⁵⁶.

2.7.4 Malignancy

CD is associated with an increased risk of overall malignancy, malignant lymphoma, and gastrointestinal malignancy (small-intestinal, oropharyngeal, esophageal, large intestinal, hepatobiliary, and pancreatic carcinomas¹⁶⁶), but reduced risk of breast cancer¹⁶⁶ and lung cancer¹⁶⁷. A recent study of biopsy-verified CD showed increased risk of GI malignancies only during the first year after CD diagnosis¹⁶⁸ suggesting that the symptoms of GI malignancy lead to CD diagnosis.

2.8 COMPLICATIONS STUDIED IN THIS THESIS

2.8.1 Study I: Venous thromboembolism

Venous thromboembolism (VTE) is a major source of morbidity and mortality worldwide¹⁶⁹. VTE has been linked to chronic inflammation (IBD)¹⁷⁰, and autoimmune disease (diabetes mellitus)¹⁷¹. Several case reports suggest a positive association between CD and VTE¹⁷²⁻¹⁷⁶. An Austrian study found no association between CD and VTE, although it was originally designed to study IBD and included only a small number of patients¹⁷⁰.

High serum levels of homocysteine is considered a risk factor for VTE¹⁷⁷⁻¹⁷⁹, although one study found no positive effect of homocysteine lowering by vitamin supplementation on the risk of VTE¹⁸⁰. Homocysteine is a sulfur amino acid that is metabolized in pathways requiring vitamin B12, folate, and vitamin B6¹⁸¹. Individuals with CD suffer a three-fold increased risk of hyperhomocysteinemia at time of diagnosis¹⁸², likely due to vitamin deficiencies. In the study by Saibeni et al, folate and homocysteine levels correlated with mucosal lesion severity, and most patients normalized vitamin levels and homocysteine levels after initiation of a GFD¹⁸². It is important to note that mucosal aberrations may persist for years in treated CD¹³⁰, and that there is evidence of vitamin B6 and folate deficiency as well as hyperhomocysteinemia even after 8-12 years of GFD¹⁴⁰. These notions support the hypothesis that hyperhomocysteinemia may cause an increased risk of VTE in CD. Other suggested mechanisms that may contribute to an increased risk of VTE in CD include high levels of thrombin-activatable fibrinolysis inhibitor (TAFI)¹⁸³ and a positive association between CD and anti-phospholipid syndrome¹⁸⁴.

2.8.2 Study II: End-stage renal disease

End-stage renal disease (ESRD), defined by the need of renal replacement therapy (dialysis or transplantation) is a severe and costly disorder. The Swedish prevalence of ESRD was 867 per million people (PMP) in 2008¹⁸⁵ and has increased to 904 PMP by the end of 2010¹⁸⁶, with a stable incidence of 125 per million person-years¹⁸⁵. In Sweden, diabetic nephropathy is the most common reason for incident ESRD, whereas primary glomerulonephritis is the most common underlying disorder in prevalent ESRD¹⁸⁵. The prevalence of ESRD is increasing worldwide¹⁸⁷. Underlying reasons are poorly understood, however, increased prevalence of diabetes mellitus is an important contributor¹⁸⁸.

Previous research has led to a suspicion that impaired oral tolerance may be associated with renal disease. Indeed, patients with primary glomerulonephritis often display an activated mucosal immune system¹⁸⁹, increased gut permeability^{190, 191}, and an increased number of mucosal intra-epithelial T-lymphocytes¹⁹⁰. Furthermore, CD autoantibodies have been observed in individuals with renal disease^{192, 193} and some renal disease will improve on a low-antigenic diet lacking in gluten¹⁹⁴.

Previous research has suggested an association between CD and renal disease^{161, 195, 196}. Collin et al showed an increased prevalence of CD among individuals with IgA nephropathy¹⁹⁵. Peters et al showed an increased mortality rate of nephritis in individuals with CD (Standardized mortality rate, 5.4)¹⁶¹, and this finding was supported by a study from our group reporting an increased risk of dialysis (HR, 3.48; 95% CI 2.26 – 5.37) and renal transplantation (HR, 3.15; 95% CI 1.29 – 7.71) in CD¹⁹⁶. These studies^{161, 196} were limited to in-patient diagnoses of CD.

2.8.3 Study III: IgA nephropathy

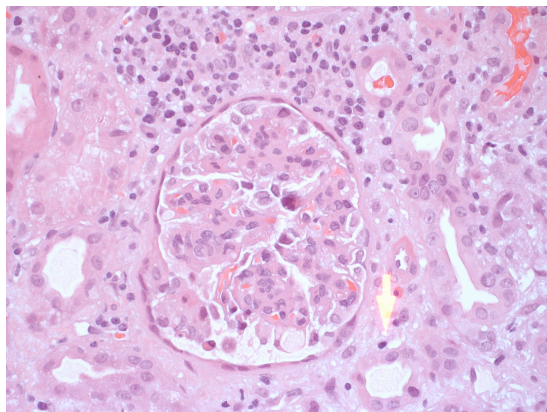
IgA nephropathy (IgAN) is an immune-complex mediated glomerulonephritis. It was first described by Berger and Hinglais in 1968¹⁹⁷. IgAN is the most common type of primary glomerulonephritis in the world¹⁹⁸, occurring in 15-40 PMP and year in Europe, with progression to end-stage renal disease in 15-50% of cases¹⁹⁹. It is the most common type of glomerulonephritis underlying ESRD in Sweden¹⁸⁶. IgAN is diagnosed through renal biopsy where the biopsy specimen is examined by light microscope, immunofluorescence (IF) or immunohistochemistry (IH) studies, and

electron microscopy. Although there is a wide range of histological abnormalities that may be present upon examination, the most common alteration identified by light microscope is an expansion of mesangial regions. IH/IF examinations should demonstrate a predominant deposition of IgA antibodies in conjunction with symptoms suggestive of IgAN to establish the diagnosis¹⁹⁹.

In IgAN, the clinical presentation may vary from asymptomatic disease to macroscopic haematuria and impaired renal function. The “classic” presentation of IgAN includes macroscopic haematuria and impaired renal function coinciding with infections of the upper respiratory tract. Although the pathogenesis of IgAN is unknown, a role for impaired oral tolerance has been implied²⁰⁰. An increased number of IELs¹⁹⁰, increased prevalence of CD autoantibodies^{192, 193, 201, 202}, and increased gut permeability¹⁹¹ have been reported in IgAN. Furthermore, improvement of parameters such as proteinuria and microscopic haematuria upon initiation of a GFD has been reported in IgAN^{194, 203, 204}.

In earlier studies assessing the association between CD and renal disease, no specific information on the presence of IgAN was available^{161, 196, 205}. In 2002, Collin et al found a high prevalence of CD (3.6%) among 223 consecutive patients with IgAN¹⁹⁵. Furthermore, previous case reports^{206, 207} and one small study²⁰² have suggested an association between CD and IgAN, however results are inconclusive²⁰⁸.

a.



b.

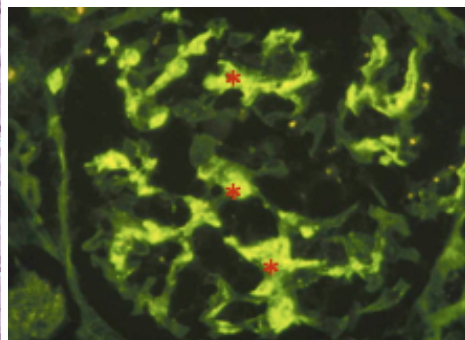


Figure 7A. (Left) Hematoxylin staining, light microscopy, renal biopsy. B. (Right) Immunofluorescence microscopy, renal biopsy. * = Mesangial IgA depositions

Pictures on this page by courtesy of associate professor Birgitta Sundelin, department of Pathology, Karolinska University Hospital, Stockholm, Sweden.

3 AIMS

The overall aim of this thesis was to achieve an increased understanding of the pathogenesis and the burden of complications in CD. CD is a common disorder worldwide, and the most common chronic disorder among children. Sweden is well suited for epidemiological studies by the use of national health data registers and national quality registers providing high quality data.

To ameliorate the understanding of disease burden in CD, the following specific aims were considered

- To explore if the risk of hospital admission due to venous thromboembolism is increased in CD;
- To assess the risk of CD among individuals with venous thromboembolism;
- To investigate the risk of renal disease (end-stage renal disease and IgA nephropathy) in individuals with biopsy-verified CD;
- To assess the risk of CD in individuals with prior renal disease.

Regarding the role of infections in CD pathogenesis, the specific aim was to

- Investigate if infection at time of gluten introduction is a risk factor for future CD.

4 SUBJECTS AND METHODS

4.1 DATA SOURCES

4.1.1 National health data registers

4.1.1.1 *The Swedish patient register*

The National Board of Health and Welfare has collected information on individual hospital discharges in Sweden since 1964. Psychiatric diagnoses were added in 1973, and complete national coverage in the Swedish hospital discharge register was reached by 1987. Validation studies have shown high specificity, with positive predictive values of about 85-95% for most diagnoses²⁰⁹. From 1997, surgical day care procedures are reported, and since 2001, all hospital-based outpatient visits have been reported to the Swedish outpatient register²⁰⁹. For every patient visit or discharge, these patient registers provide information regarding diagnosis and surgical procedures according to the International classification of diseases (ICD).

The Swedish patient register was used to identify exposure (study I) and outcome measures in study I (Swedish hospital discharge register) and study II. It was also used to identify covariates in studies I-III, and prior renal disease in studies II and III.

4.1.1.2 *The total population register*

The Swedish total population register (TPR) is kept by Statistics Sweden. It contains data on basic demographics such as the personal identity number, sex, age, civil status, area of residence, country of birth and date of emigration. Information is collected and continuously updated by local tax offices. The register was computerized in 1968²¹⁰. We used the TPR to select matched reference individuals and to collect data for censoring (emigration date or death) in studies I-III.

4.1.2 The Swedish renal register

The Swedish renal register (SRR) is a national quality register originally established in 1991. Individuals with renal replacement therapy (dialysis or transplantation) are reported to the register, and all Swedish renal treatment units participate. In a validation study, coverage was reported at >95%²¹¹.

We used the SRR to validate the outcome (ESRD) in study II, and to access data on underlying disease causing renal failure.

4.1.3 The educational register

Information regarding educational level (seven pre-defined levels) was obtained through Statistics Sweden. In children, the education level of the parents is recorded. Data on education level are used as a proxy for socioeconomic position. Missing data could concern people with degrees from abroad or with private degrees or with internal company-educations, or people with old degrees.

4.1.4 The Swedish prescribed drug register

The Swedish prescribed drug register contains all prescribed drugs dispensed to Swedish residents at pharmacies in Sweden since July 1, 2005, and has an almost 99% coverage²¹². Data from the register were used to identify individuals for whom antihypertensive agents were dispensed in study II.

4.1.5 Small intestinal biopsy data

Small-intestinal biopsy is the golden standard of diagnosing CD, performed in at least 9/10 cases by 100% of Swedish pediatricians and by 96% of Swedish gastroenterologists prior to diagnosing CD¹²⁰. Data from small intestinal biopsies performed between July 1969 and February 2008 were collected between October 27, 2006, and February 12, 2008. This was achieved through computerized searches of all (n=28) pathology departments in Sweden. Searches were carried out by local technicians and rendered data on the arrival date of biopsy material, personal identity number, morphology according to SnoMed classification codes (villous atrophy, M58) and topography (duodenum/jejunum). CD was defined as VA (equal to Marsh stage III) of the duodenum or jejunum. 29,096 individuals of biopsy-verified CD were identified. The mean age at first recorded small intestinal biopsy with VA was 30 years. The majority of individuals were diagnosed in adulthood and after 1990.

4.1.6 Renal biopsy data

Renal biopsy examination by light microscopy and immunofluorescence or immunohistochemistry has been the gold standard for IgAN diagnosis in Sweden since the 1970s. Computerized records are available since the early 1990s in all of Sweden. Renal biopsies are assessed at four pathology departments in Sweden (Stockholm, Gothenburg, Linköping, and Malmö/Lund). Individuals with IgAN were identified by computerized searches in all four departments, using relevant SnoMed coding (IgAN, D67300). In one department (Malmö/Lund), coding had not been registered. Therefore, data from all renal biopsies from this department were obtained and manually scanned (AW and BS). Individuals from this region were considered to suffer from IgAN only when the pathologist's report described findings consistent with IgAN and the pathologist clearly stated IgAN as the primary diagnosis. We collected data on 4,069 unique individuals with a biopsy record of IgA nephropathy in Swedish computerized biopsy registers.

4.1.7 The ABIS study

Infants born in Southeast Sweden between October 1, 1997, and October 1, 1999, were invited to participate in the ABIS (All Babies in Southeast Sweden; the counties of Öland, Småland, Blekinge and Östergötland) cohort project. This project was launched to examine the role of environmental factors in the development of immune-mediated disease. Of the 21,700 infants born during the study period, parents of 17,055 gave informed consent for participation. 16,286 mothers completed an initial birth questionnaire at the maternity ward or at home. Parents were asked to keep a diary during the child's first year of life, providing data on factors such as diet (date of cessation of breastfeeding and gluten introduction) and infectious diseases (date of infection). These diaries were filled out prospectively at home by the parents of 9,849 children. We restricted our study to 9,408 children for whom complete data on breastfeeding duration and gluten introduction were provided by the diaries.

The majority of children with CD in the ABIS cohort were identified through a study on symptoms and signs in CD¹⁰¹. In this study, all eight pediatric departments undertaking small intestinal biopsies in children from the ABIS area registered children with CD. CD was defined as VA upon small-intestinal biopsy and (1) resolution of all symptoms after introduction of a GFD and/or (2) no or only minor histopathologic abnormalities of CD in the biopsy taken during treatment with a GFD. The ABIS population was not actively screened for CD. In 2007–2008, these pediatric departments were again contacted and asked to report on children in the ABIS study with biopsy-verified CD (VA). CD-consistent symptoms and antibody markers were required for the diagnosis of CD. Data collection in the ABIS study is described in Figure 8.

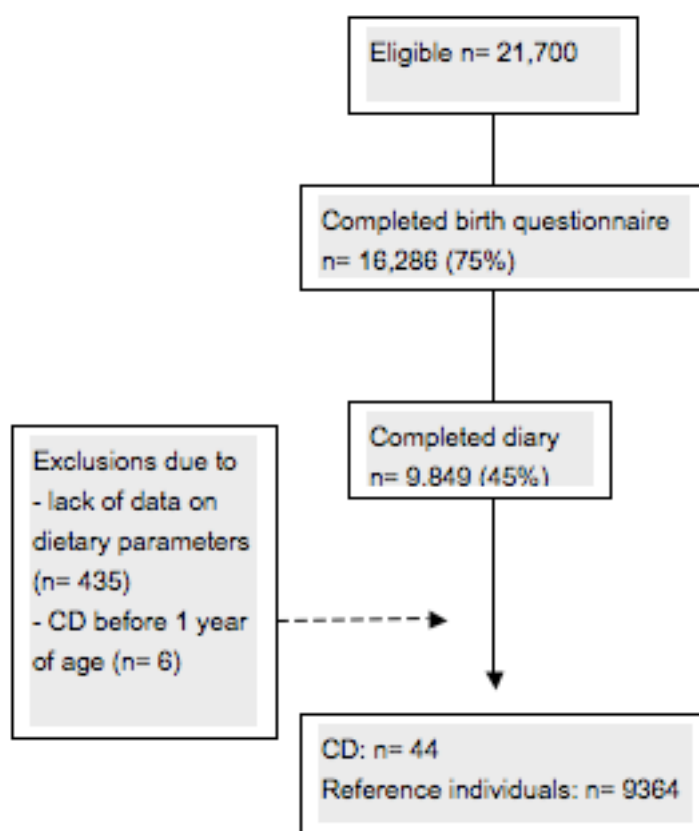


Figure 8. Data collection in the ABIS study

4.1.8 The Swedish personal identity number

The Swedish personal identity number (PIN) is an important tool that enables linkage of data from Swedish health data registers. Since 1947, the National Tax Board has assigned a ten-digit PIN at birth or immigration to all individuals that have resided in Sweden. The first six numbers are the date of birth (year-month-day), followed by three digits identifying the individual and a last digit (control digit). The PIN is used by all health care providers in Sweden, allowing virtually 100% coverage of the Swedish health care system²¹³.

4.2 STUDY DESIGN

4.2.1 Study I

This study is a population-based prospective cohort study. Individuals with CD (n= 14,207) were identified in the Swedish hospital discharge register (Figure 9). Five reference individuals, matched for age, sex, calendar year and county at the time of CD diagnosis, were selected for each individual with CD using the TPR (n= 69,048).

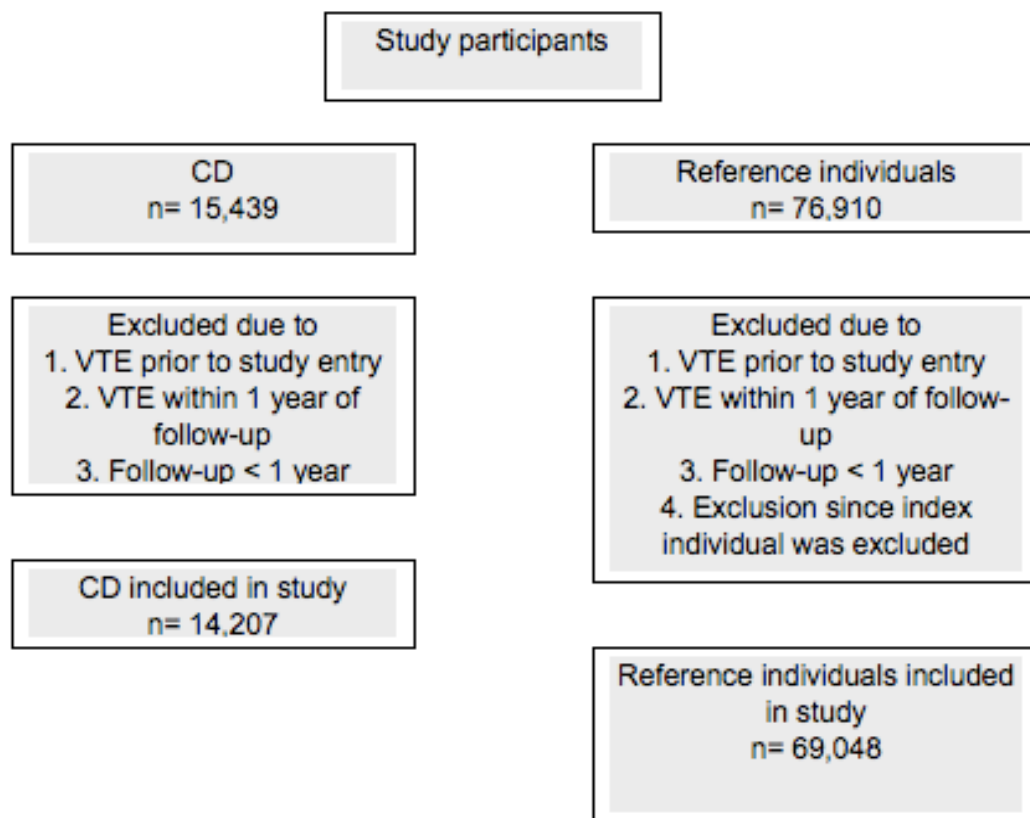


Figure 9. Data collection in study I.

Follow-up started 1 year after CD diagnosis, to minimize the risk of detection bias due to hospital admittance in CD, and ended at death, emigration, date at first discharge diagnosis of VTE or 31st of December, 2003, whichever came first. The risk of future VTE in individuals with CD was calculated using Cox regression. Analyses were performed stratum wise, which means that each individual with CD was only compared with his/her reference individuals. In separate analyses, we stratified by age (≤ 15 years vs. > 15), and sex. Diabetes mellitus is a potential confounder, being positively associated with VTE¹⁷¹, and with CD³⁶. We therefore adjusted for diabetes mellitus in a separate analysis. Additionally, we adjusted for socio-economic index since this might be associated with the predisposition of seeking medical care.

Lastly, we performed a retrospective case-control study based on the aforementioned cohort. Conditional logistic regression was used to estimate the association between CD and prior VTE. To avoid detection bias, those with one year or less between VTE and study entry (CD diagnosis) were excluded.

To determine whether a potential risk increase for VTE is specific for CD or associated with surveillance bias, post hoc analyses were performed restricting reference individuals to individuals with a record of being inpatients within 1 year prior to or after the index case with CD. This analysis was not internally stratified due to the fact that many reference individuals were excluded; instead we adjusted for age, sex and calendar period.

4.2.2 Studies II and III

These studies are population-based prospective cohort studies. Individuals with CD were identified through Swedish biopsy registers (see above). Study entry was defined as date of CD diagnosis (biopsy with VA) or corresponding date in reference individuals. Outcome measures (ESRD or IgAN) were identified using the Swedish patient registers and the SRR (study II) and a database of biopsy-verified IgAN collected from Swedish pathology departments (study III). Up to five reference individuals per index person matched for age, sex, calendar period and county, were identified using the TPR. We excluded individuals for whom patient registers or the SRR indicated ESRD prior to study entry (study II) and individuals with any record of renal disease in patient registers or biopsy with IgAN prior to study entry (study III). Data linkage in studies II and III is described in Figure 10.

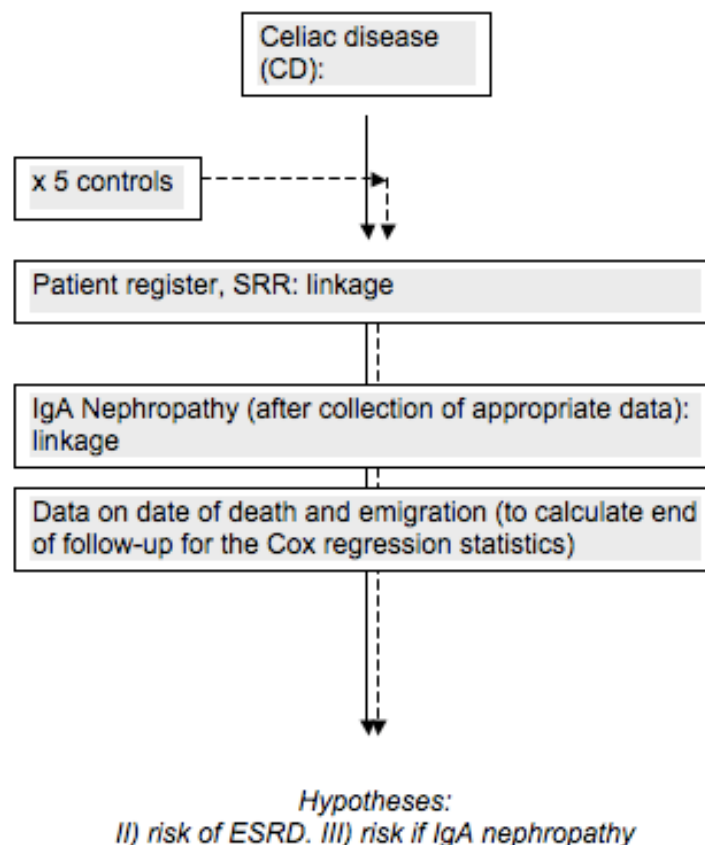


Figure 10. Data linkage in studies II and III.

Follow up ended at date of ESRD/IgAN diagnosis, emigration, death or the 31st of December 2008, whichever came first. Analyses were performed stratum-wise (each individual was

performed only with his/her controls).

Diabetes mellitus type 1 is associated with CD³⁶ and renal disease and is thus a potential confounder to be considered in studies II and III. Because early versions of Swedish ICD coding (ICD7-9) do not distinguish between type 1 and type 2 diabetes, we defined diabetes mellitus as a diagnosis of diabetes reported to the Swedish patient register before the age of 30 years. Liver disease is positively associated with CD²¹⁴ but may also cause renal IgA deposits (secondary IgAN)²¹⁵. We therefore adjusted for liver disease in study III, obtaining information on this covariate from the Swedish patient register. Analyses performed in studies II and III are described in Table 7.

Table 7. Analyses performed in studies II and III.

	Study II	Study III
	CD n= 29,050 Reference individuals n= 144,363	CD n= 27,160 Reference individuals n= 133,949
<i>Stratified analyses</i>	Age Sex Calendar period Follow-up	Age Sex Calendar period Follow-up
<i>Adjusted analyses</i>	Diabetes mellitus type 1 Education level Country of birth Any renal disease prior to study entry	Diabetes mellitus type 1 Education level Country of birth Liver disease
<i>Additional analyses</i>	Risk of subsequent CD in individuals with ESRD Outcome restricted to ESRD in patient register AND in the SRR Adjustment for use of any hypertensive drugs	Risk of subsequent CD in individuals with IgAN

In both studies, we performed additional case-control studies assessing the risk of future CD in individuals with ESRD or IgAN. This was done using conditional logistic regression, with study entry at time of ESRD/IgAN and the end of study at first diagnosis of CD. These analyses were performed to evaluate whether the association between CD and the outcome is restricted to patients with CD preceding the outcome, thus providing further information on the temporal sequence of the association (see Figure 11). If the primary analysis shows a positive association between CD and future IgAN/ESRD (black arrow) this suggests that the characteristics of CD increase the risk of IgAN/ESRD. If, concomitantly, the risk of future CD in IgAN/ESRD is elevated, this suggests shared genetics or environmental factors in the two disorders (red arrows).

If, however, the risk of future CD in IgAN/ESRD is elevated, but not the reverse, this suggests that the characteristics of IgAN increases the risk of CD (green arrow).

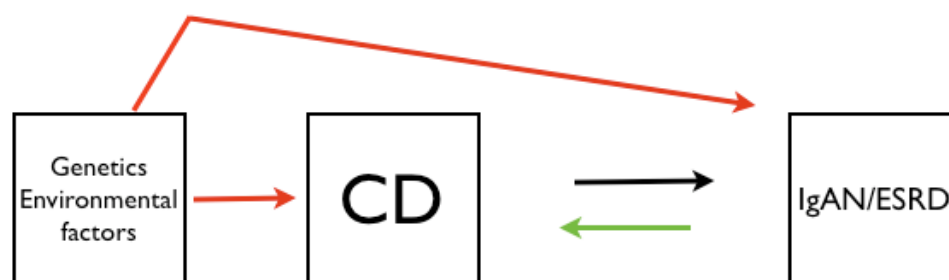


Figure 11. Potential flow of associations in studies II and III.

In a separate analysis we examined the risk of ESRD in prior CD adjusting for hypertension. Since the sensitivity for hypertension is low in the Swedish hospital discharge register²¹⁶, we used anti-hypertensive medication as a proxy for hypertension. Data on such medication were obtained through the Swedish prescribed drug register. We used the ATC codes (Anatomical, Therapeutical and Chemical classification system) C02, C03 C07 and C08 to identify individuals with hypertension (anti-hypertensive medication). In a post hoc subanalysis we then adjusted for antihypertensive medication in patients with follow-up until at least July 1, 2005, when the Swedish prescribed drug register started.

4.2.3 Study IV

This is a prospective cohort study. Using data from the ABIS cohort, we calculated the risk of future CD in children with any infectious disease or gastroenteritis at time of gluten introduction, adjusting for age at gluten introduction and age at end of breastfeeding. The reference group consisted of children from the ABIS cohort for whom dietary data were completed and no diagnosis of CD was observed. After discussions regarding the incubation periods of different infectious diseases and their potential effects on the immune system (A. Ternhag, MD, PhD, personal verbal communication, 2008) time of gluten introduction was defined as 3 weeks prior to gluten introduction or in the week immediately following gluten introduction (see Figure 10). We defined gastroenteritis as a parent-reported episode of repeated vomiting, diarrhea, or stomach flu. Follow-up started at one year of age, to avoid potential recall bias. Follow-up ended on June 30, 2006 or date of CD diagnosis, or death.

Data were analyzed in several steps. Initially, we compared infectious load during the first year of life in children with and without CD using the Mann Whitney U-test. Univariate analyses included stratification for age initially exposed to gluten, age at end of breastfeeding, and age at any infection or gastroenteritis. Bivariate analyses included analyses adjusted for age initially exposed to gluten and age at any infection, and age at end of breastfeeding. In our final, multivariate analysis, we tested the association between future CD and parent-reported infection (any infection or gastroenteritis) at time of gluten introduction (Figure 12) whilst adjusting for age at end of breastfeeding, age at gluten introduction, and age at any infection (or gastroenteritis).

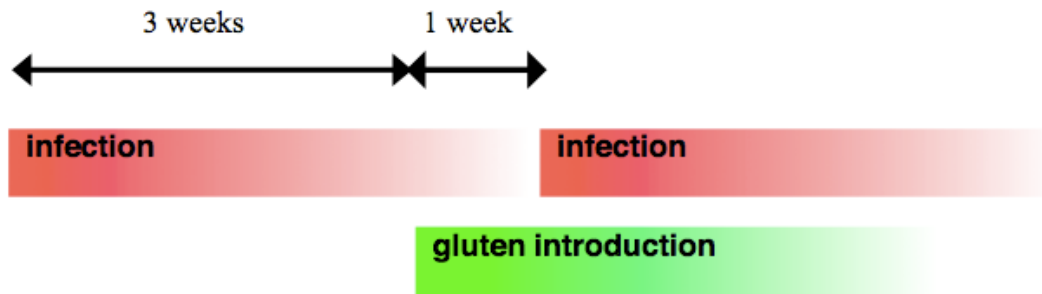


Figure 12. Infection at time of gluten introduction.

4.3 STATISTICAL METHODS OF RELEVANCE

4.3.1 Cox proportional hazards model (studies I-IV)

David R. Cox first proposed the Cox proportional hazards model in 1972²¹⁷. It has since become the most commonly used statistical model applied in medical time-to-event studies²¹⁸. A classic example of a time-to-event study is the study of survival time after a sombre diagnosis. The Cox proportional hazards model is constituted of a baseline hazard function ($h_0(t)$) and an exponential function (see Equation 1). The baseline hazard function is unspecified (non-parametric), whereas the exponential function is completely specified, apart from the unknown parameters (parametric); thus the full Cox model is considered semi-parametric. The Cox model assumes proportional hazards, i.e. that the difference in risk between groups is constant over time.

$$h(t | x_1, x_2, \dots, x_k) = h_0(t) \exp(\beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k)$$

Equation 1. The Cox proportional hazards model

The output of interest in Cox modeling is the hazard ratio (HR). The hazard is the risk of the outcome, for example death, at a given time interval $t + \Delta t$ given survival to time t . The hazard ratio is the relative hazard comparing two groups, assuming that they meet the criteria of proportional hazards. If this criterion is not met, estimated HRs will be misleading.

We used Cox regression to estimate risk of CD in study IV, and to estimate the risk of subsequent complications in individuals with CD in studies I-III. For these studies, we used an internally stratified approach (each individual with CD was compared with his/her matched reference individual prior to pooling of the estimates). Log-minus-log curves showed that the proportional hazards assumption was valid (example curve, see Figure 13).

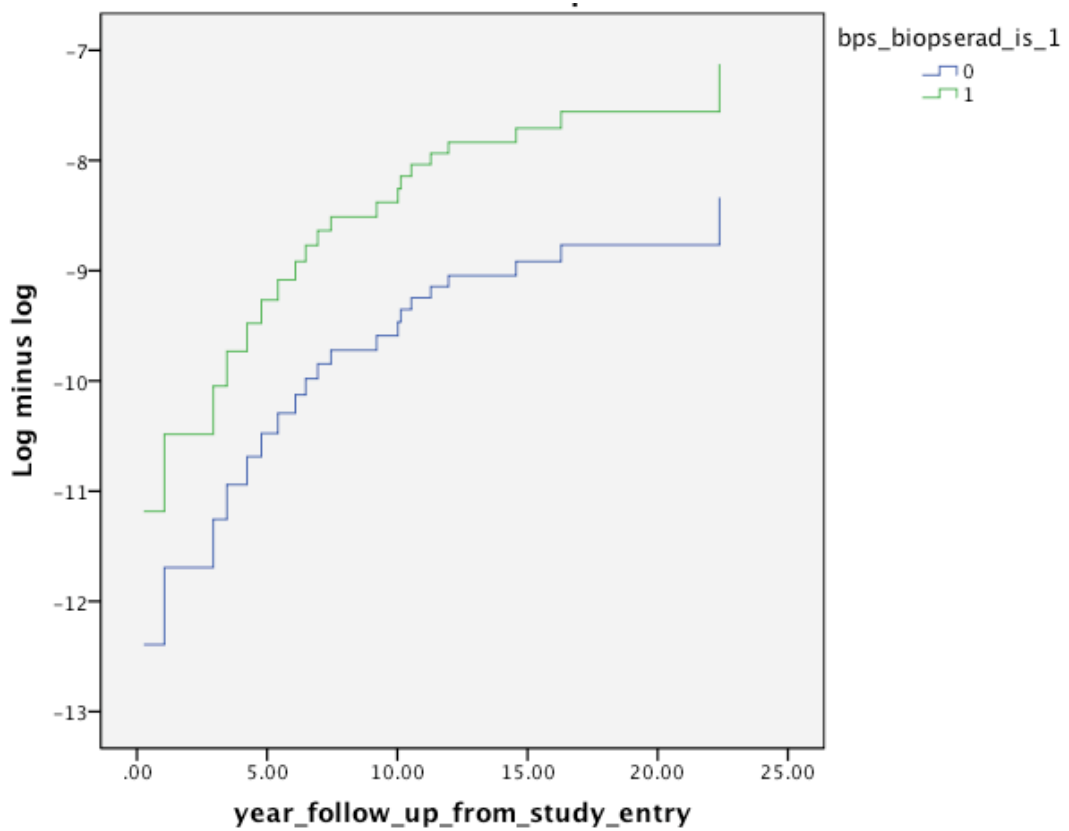


Figure 13. Log-minus-log curve, study III.

4.3.2 Logistic regression (studies I-III)

Logistic regression is used to model the association between one or several independent variables (covariates) and a binary outcome variable. With a binary (or dichotomous) outcome variable, what is modeled is the logit transformation of the odds (π) of the outcome given x (Equation 2).

$$\pi(y | x) = \{e^{\beta_0 + \beta_1 x} / (1 + e^{\beta_0 + \beta_1 x})\}$$

$$\ln(\pi(x) / (1 - \pi(x))) = \beta_0 + \beta_1 x$$

Equation 2. Logistic regression model and logit transformation.

This transformation is important and desirable, since it shares properties with the familiar linear regression model. It is continuous and linear, with the potential for a range of $-\infty$ to $+\infty$, given x . The basis for the model is the method of maximum likelihood, whereby advanced software produces estimates for the coefficients that maximize the probability of obtaining the observed set of data²¹⁹.

Logistic regression makes a few assumptions. Firstly, each observation must be independent. Multicollinearity is not tolerated (independent variables must be independent from each other). Logistic regression does not require the independent

variable to be linearly correlated with the dependent variable. It does, however, require linearity between the independent variable and the logit.

An intuitive interpretation of risk is to consider the probability (p) of disease in a population. Another way to calculate risk is to divide the number of patients who contracted the disease over the number of patients who *did not*, producing the odds of the event ($p/(1-p)$). The output of interest in logistic regression is the odds ratio (OR), which can be calculated from the estimated coefficient β_1 . The odds ratio is defined as the cases' odds of having been exposed to a certain risk factor (x), divided by the reference individuals' odds of having been exposed to the same risk factor. Note that the probability (p) of y given x (Equation 2) can be reduced to $p/(1-p)$.

4.3.2.1 Conditional logistic regression

In conditional logistic regression, the association between being a case or not being a case and a set of prognostic factors is assessed stratum-wise. This is often used in matched case-control studies. The difference from regular logistic regression is that each individual is compared only with his/her matched cases, thus minimizing the effect of the matching variables. An option could be to use unconditional logistic regression and instead include the matching variables in the analysis. This, however, will lead to an overestimation of the OR.

We used conditional logistic regression in nested case-control studies in studies I-III, when we assessed the risk of subsequent CD following VTE/ESRD/IgAN. In these studies, time-to-event was not considered.

4.4 ETHICAL CONSIDERATIONS

Study I: This project (04-030/1) was approved by the Research Ethics Committee of the Karolinska Institute, Stockholm, Sweden on the 18th of March 2004.

Study II: This project was approved by the Research Ethics Committee of the Karolinska Institute, Stockholm, Sweden on the 31st of April, 2006 (dnr 2006/633-31/4).

Study III: This project was approved by the Research Ethics Committee of the Karolinska Institute, Stockholm, Sweden on the 31st of April, 2006 (dnr 2006/633-31/4). Additions to this project is also described and approved by the same committee (dnr 2010/407-31/1).

Study IV: This study is part of the ABIS project, which was originally approved by the Research Ethics Committees of the Faculty of Health Sciences, University of Linköping (Li 287-96) and the Medical Faculty, University of Lund (Lu 83-97).

Studies I-III are register-based cohort studies, based on information independently collected from patients as part of a standardized process. Given the temporal volume of data collected in national registers, a substantial number of individuals included in these studies are likely dead. The ethics committee requested us not to contact any participants of these studies, since this would likely cause more harm than patient benefits. Abstaining from informed consent is customary in Swedish large-scale register based studies. All data were made anonymous prior to analysis to protect patient privacy.

5 RESULTS

5.1 COMPARING THE COHORTS (STUDIES I-III)

There are some differences between the CD cohort characteristics in study I vs. studies II-III that are of value to consider when interpreting the results. Briefly, individuals with CD in study I were median 2 years of age at CD diagnosis, compared with a median age of about 30 years of age in the biopsy cohort. Furthermore, the majority of individuals with CD in study I were diagnosed before 1994 (74.9%), whereas 85.9% were diagnosed after 1990 in studies II-III. In studies I-III, CD was more common in women (ratio of circa 6:4), and type 1 diabetes mellitus occurred more frequently in individuals with CD compared with reference individuals. This implies that studies II-III investigates a more modern CD cohort, with a greater number of individuals diagnosed with CD in adulthood. These characteristics likely reflect the increased use of CD serology over time, and increased awareness that CD may present in adulthood.

5.2 CD AND VENOUS THROMBOEMBOLISM (STUDY I)

Of 15,439 individuals with CD, 406 (2.6%) suffered subsequent VTE, compared with 1105/76,910 (1.4%) of reference individuals. We found a modestly increased risk of future VTE in individuals with CD (HR, 1.86; 95% CI 1.54-2.24). This risk increase was seen in men and in women, and remained significant when the first year of follow-up was included. Stratified analyses showed no increased risk of VTE in individuals diagnosed with CD before the age of 16, however heterogeneity testing suggested no significant difference in risk according to age at CD diagnosis ($p=0.072$). The estimate remained significant in analyses adjusted for diabetes mellitus and socioeconomic index. No difference in risk of VTE was seen with respect to sex ($p=0.739$) or age ($p=0.072$). The risk increase was lower when we restricted reference individuals to inpatients (HR, 1.27; 95% CI 1.06-1.52).

Conditional logistic regression revealed increased risk of subsequent CD in individuals with prior VTE (HR, 1.67; 95% CI 1.34-2.06).

5.3 CD AND END-STAGE RENAL DISEASE (STUDY II)

Among 29,050 individuals with CD, 90 (0.3%) developed ESRD, compared with 152/144,363 (0.1%) in reference individuals. This corresponds to an incidence rate of ESRD in individuals with prior CD of 30/100,000 person-years, and 10/100,000 person-years in reference individuals.

We found a nearly three-fold increased risk of future ESRD in individuals with prior CD (HR, 2.87; 95% CI 2.22-3.71). The most common underlying renal disease in individuals with CD and in reference individuals was diabetes nephropathy. The estimate remained significant in analyses adjusted for the presence of diabetes mellitus type 1, education level, or country of birth (Nordic vs. non-nordic). Excluding individuals with records of any renal disease prior to CD diagnosis had little effect on the estimate (HR, 2.47; 95% CI 1.80-3.40). When we restricted ESRD to individuals with a record of ESRD in a patient register *and* in the SRR, the risk estimate was slightly elevated (HR, 3.06; 95% CI 2.34-4.01). Upon stratification for modality of

treatment, we found an increased risk of dialysis (HR, 3.06; 95% CI 2.34-4.01) but not renal transplantation (HR, 1.55; 95% CI 0.67-3.62) in individuals with CD.

Separately, we examined the association between prior ESRD and CD using conditional logistic regression (case-control design). Individuals with prior ESRD suffered an increased risk of later CD (Odds ratio (OR), 2.45; 95% CI 1.74-3.44).

5.4 CD AND IGA NEPHROPATHY (STUDY III)

Of 27,160 individuals with CD and no prior renal disease, 7 developed IgAN (0.03%), corresponding to an incidence of 25 PMP and year. Among reference individuals, the incidence rate was 8 PMP and year (11 cases of IgAN, 0.008%). We found an increased risk of biopsy-verified IgAN in individuals with biopsy-verified CD (HR, 3.03; 95% CI 1.22-7.56). Although interaction tests revealed no difference in risk of IgAN according to sex ($p=0.32$), only one woman developed IgAN and stratified analyses showed increased risk for future IgAN only in men. Due to lack of power, analyses stratified by age at CD diagnosis, year of CD diagnosis and length of follow-up yielded increased HRs but wide confidence intervals including 1, or could not be calculated due to a lack of positive events. No individuals suffered from diabetes mellitus type 1. The risk estimate remained increased after adjusting for country of birth (HR, 2.96; 95% CI 1.19-7.37) and education (HR, 2.73; 95% CI 1.03-7.26). The risk estimate remained significantly increased after adjusting for any liver disease (HR, 3.19; 95% CI 1.27- 8.06).

We used conditional logistic regression (case-control design) to calculate odds ratios (ORs) to assess the association between prior IgAN and CD. We found no increased risk of future CD in individuals with IgAN (OR, 1.70; 95% CI 0.70-4.12 (data adjusted for age, sex, and calendar year).

5.5 INFECTION AT TIME OF GLUTEN INTRODUCTION AND RISK OF FUTURE CD (STUDY IV)

At end of follow-up (Dec 1st, 2006), study children were aged 8 years and there were 50 cases of CD, corresponding to a prevalence of diagnosed CD of 1:188 children (0.53%). Six of the children with CD received their diagnosis during their first year of life and were therefore excluded from the main analyses. 92.4% of children had at least one parent-reported infection during their first year of life (average number of infections, $n=4.6$). In total, we had data on 42,826 episodes of infectious disease, reported with date of onset. 34.1% of children had at least one episode of parent-reported gastroenteritis during their first year of life. The follow-up time in the 9,408 children (CD $n=44$), starting at age 1 year, was 68,206 person-years. Except where explicitly stated, all analyses were restricted to these 9,408 children.

5.5.1 Breastfeeding duration, introduction of gluten and risk of CD

No individual with CD had received gluten in the first three months of life, as compared to 1.1% of reference individuals. Almost half of participating children were breastfed for more than 10 months. Gluten was most often introduced during months 5 and 6. Age at gluten introduction or end of breastfeeding was not associated with future CD.

5.5.2 Any infection, gastroenteritis and risk of CD

Infection at time of gluten introduction was associated with an increased risk of future CD (HR, 1.9; 95% CI 1.0-3.4; P = 0.038), while gastroenteritis at time of gluten introduction was not (HR, 1.3; 95% CI 0.2-9.4) (unadjusted analyses). In a final model, adjusted for age at gluten introduction, age at end of breastfeeding and age at infection (or gastroenteritis), we found no statistically significant association between infection at time of gluten introduction and future CD (adjusted HR, 1.8; 95% CI 0.9-3.6; p = 0.111). Additionally, gastroenteritis at time of gluten introduction was not significantly associated with increased risk of future CD (adjusted HR, 2.6; 95% CI 0.2-30.8; p = 0.439).

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Study design

6.1.1.1 Cohort studies (I-IV)

Randomized clinical trials are considered the standard of excellence with the highest degree of evidence for evaluations in health care²²⁰. Experimental studies may, however, not be always be feasible nor appropriate. Observational studies are thus warranted in medical research. Epidemiological studies are cohort studies, case-control studies, or variations of the two. Choosing between the two is generally a trade-off between validity and efficiency.

In cohort studies (from the Latin cohorts, plural cohortes, used to describe a military unit) groups of individuals sharing similar pre-defined exposure types (e.g. CD and reference individuals) are followed over time and the incidence rate of the outcome of interest is compared between the different cohorts yielding estimates of relative risk²²¹. Cohort studies are generally considered to have a higher validity than case-control studies, suitable for studies where the exposure of interest is rare, and practical since they provide estimates of absolute risk. They are, however, often expensive and time consuming. Although these disadvantages may be true for some cohort studies, historical cohorts based on national registers have different characteristics. Swedish national registers contain data that are prospectively collected, minimizing the risk of recall bias. Data are registered in different registers without knowledge of planned studies, a practice that minimizes the risk of biased registrations. The time consumption is reduced since the cohorts are already initiated and outcomes registered. Additionally, the costs of these studies are independent of the study size. A disadvantage with historical cohort studies is that data collection rarely is designed with regards to the studies being undertaken. Thus, a lack of detail may limit availability of exposure data and information regarding potential confounders.

6.1.1.2 Case-control studies (I-III)

Case-control studies are a cost-efficient approach to study the relationship between exposure and outcome in epidemiological studies. This design is also suitable when the outcome of interest is rare, and a cohort study would be inefficient. Cases are individuals that develop the outcome, and the controls do not. Comparing the frequency of exposure in the two groups in a retrospective manner enables assessment of whether the exposure is a risk factor for the outcome. The validity of a case-control study relies heavily on the comparability of cases and controls. Since only a sample of the study base is collected, absolute risks and incidences rates cannot be calculated in a case-control study. The output of interest is commonly the odds ratio (OR), defined as the odds that a case is exposed divided by the odds that a control is exposed. The interpretation of the OR is analogous to the RR obtained in cohort studies, but will overestimate RRs > 1 and underestimate RRs < 1 when the outcome becomes more frequent²²².

6.1.2 Internal validity

In the context of epidemiological studies, internal validity is the extent to which the study de facto measures what it set out to do, i.e. how the inferences drawn pertain to the subjects that are under study. In contrast, external validity is concerned with the generalizability of study results, i.e. how inferences drawn relate to individuals outside the study population²¹⁸. Two types of errors threaten the internal validity; random error and systematic error (bias).

6.1.2.1 Recall bias

Recall bias is the effect of prior knowledge regarding outcome status affecting retrospective reports of exposure status, and is a common issue in case-control studies. Recall bias makes cases and controls less comparable, since cases may be more prone to recall exposure than controls (increasing sensitivity among cases) or more likely to construct false memory of exposure (reducing specificity among cases), or the disease itself may cloud the memory.

In contrast to earlier studies on infant feeding practice and CD⁸⁵⁻⁸⁷, exposure data in study IV were collected prospectively in an attempt to minimize recall bias. We cannot exclude that for some children, parents delayed diary completion and filled it in retrospectively at the end of the child's first year of life. At this time, although not yet having received a diagnosis of CD, initial disease symptoms may have increased the awareness and carefulness of diary completion among parents, introducing potential recall bias however to a presumably low extent.

6.1.2.2 Selection bias

Selection bias refers to biases that result from the procedure of selecting study participants and from factors that influence participation. Common examples are the healthy worker effect²²³, volunteer bias, inappropriate selection of controls in case-control studies, and differential loss to follow-up. In brief, due to selection bias the groups under study are not comparable and the association between exposure and disease is different for those included in the study compared with the underlying study base.

In study I, exposure status was determined by data from the Swedish hospital discharge register. This imposes a risk of selection bias, since individuals requiring hospital admission may have a more severe disease than the average individual with CD. This selection bias would be more pronounced if all individuals were diagnosed today, but in the study most individuals were identified in the 1980's and 1990's when hospitalization was more common. Additionally, the median age at study entry was two years. Since young children often need anesthesia for biopsy acquirement, hospitalization would be more common in this group. We therefore believe that this cohort is largely representative of the celiac population during the study period.

To avoid selection bias due to hospitalization, we used national biopsy registers to assess exposure status in studies II-III. A differential degree of outcome registration (VTE, ESRD, IgAN) due to exposure status (CD) cannot be ruled out but seems unrealistic. Due to the use of national registers to sample reference individuals, all Swedish residents have the same probability of being included, provided they had no record of an earlier small intestinal biopsy.

Although the ABIS cohort is population based, the analyses were restricted to children whose parents chose to participate in the study and for whom the diary was sufficiently completed. We cannot rule out that participating parents were more prone to comply with dietary guidelines and that their infant's risks of CD subsequently would differ from the general population. Since >95% of diaries contained complete data on breastfeeding and gluten introduction, we consider the risk of selection bias due to diary completion very low.

6.1.2.3 *Detection bias*

Detection bias, or surveillance bias, is common in epidemiological studies and arises when exposure leads to increased or decreased likelihood of observing the outcome. In study I, we cannot exclude that individuals with CD admitted to a hospital suffer increased risk of the outcome due to increased awareness of symptoms and check-up visits that might lead to the diagnosis of a VTE. To minimize the risk of detection bias, we excluded individuals with follow-up less than 1 year in our main analysis. Additionally, we performed separate analyses restricting our reference individuals to inpatients.

We cannot rule out that part of the risk increase observed in study II is caused by surveillance bias. Since a significantly increased risk of ESRD remained even after 5 years after CD diagnosis, such bias is unlikely to explain all of the risk increase observed. Surveillance bias might inflate the risk estimate, since increased risk of future dialysis (which implies frequent blood sampling and physician visits) but not renal transplantation was observed.

6.1.2.4 *Misclassification*

Information bias, or misclassification, is defined as an error in measurement of the exposure or outcome. Differential misclassification occurs when the error depends on the other variables (i.e. misclassification of exposure depends on outcome) whereas non-differential misclassification arises when there is a measurement error of exposure/outcome that is independent of the other variables. Differential misclassification may lead to under- or overestimation of the associations in observational studies. Non-differential misclassification biases any risk estimate towards the null value, obscuring any real differences²¹⁸. Recall bias or detection bias are two examples of differential misclassification.

6.1.2.4.1 *Misclassification – discharge diagnoses of CD and VTE (study I)*

Although no study has assessed the validity of the discharge diagnoses of CD or VTE in the Swedish hospital discharge register, validation studies have confirmed a high overall quality, with positive predictive values of about 85-95% for most diagnoses²⁰⁹. In a small patient chart review, the positive predictive value of CD in the Swedish hospital discharge register was 77%, but this study was limited to patients diagnosed with CD mostly in old age¹⁵¹. A high specificity for CD is not surprising since prior small-intestinal biopsy preceding diagnosis has been required since 1969.

Considering the sensitivity of CD in the hospital discharge register, it is likely not as high as the specificity, however we believe that we have included a considerable portion of Swedish individuals with diagnosed CD. The reference population likely

includes some false-negatives given that undiagnosed CD is common²²⁴, however not to an extent that is likely to affect the estimate given a prevalence of CD of 1% on the population level. Sweden has roughly 9 million inhabitants. An earlier Swedish study of *diagnosed* CD showed a prevalence of about 1/1000²²⁴. We included 14,207 individuals with CD in the current study.

6.1.2.4.2 Misclassification- diagnosis of CD in Swedish biopsy registers (study II and III)

A recent validation study confirms that small intestinal biopsy indeed remains the gold standard in diagnosing CD. 96% of Swedish gastroenterologists and 100% of Swedish paediatricians report that they perform a small intestinal biopsy in at least 9/10 individuals prior to diagnosing CD¹²⁰. It is therefore likely that we have identified the vast majority of cases of *diagnosed* CD in Sweden. There will likely exist false-negatives among the reference individuals, however to a low extent given the prevalence of the disease.

All that is flat is not VA, however. The same validation study examined a subset of biopsy reports and found that conditions other than CD was rare in VA¹²⁰. Among 114 patients with VA, 95% had a clinical diagnosis of CD. The specificity of CD in VA is thus high.

6.1.2.4.3 Misclassification- diagnosis of ESRD (study II)

Validation studies have confirmed a high overall quality of diagnoses in the Swedish hospital discharge register, with positive predictive values of about 85-95% for most diagnoses, which renders little risk of misclassification of ESRD²⁰⁹. In individuals with CD, increased contact with health care and work-up including blood samples may lead to increased recognition of renal disease. We therefore conducted an analysis where we restricted the outcome to ESRD reported in the patient register *and* in the Swedish Renal Register (SRR), to minimize the risk of misclassification of disease (i.e. increase the sensitivity of ESRD in our study). The risk estimate remained significant after this restriction.

In our study, we report an incidence of 100 PMP among reference individuals. This number is similar to what has been reported on a national level (125 PMP¹⁸⁶), which further supports appropriate classification of outcome.

6.1.2.4.4 Misclassification- diagnosis of IgAN in Swedish biopsy registers (study III)

No previous studies have assessed the quality of the IgAN diagnosis in Swedish biopsy registers. We cannot exclude the possibility of misclassification of disease. Although renal biopsy is required for a diagnosis of IgAN¹⁹⁹, the procedure is invasive and entails a risk of bleeding and renal damage. Biopsy may be avoided in cases of mild renal disease²²⁵, resulting in a risk of false-negatives among reference individuals. Since IgAN is uncommon, the effect of these false-negatives on the risk estimate is presumably low. It is therefore likely that the specificity of IgAN in the current study is high, but that the sensitivity is lower, with implications for external validity (see section 6.2.3). The incidence rate in this study (25 PMP and year in the CD group, 8 PMP in reference individuals) was slightly lower than reported by other European studies (15-40 PMP and year), further supporting this notion.

6.1.2.4.5 Misclassification- diagnosis of CD in the ABIS study (Study IV)

Children from the ABIS cohort were not screened for CD, thus all reported cases of CD represent *diagnosed* CD (VA, CD consistent symptoms and positive CD serology). The occurrence of false-negatives among reference individuals is thus likely but presumably with little effect on the estimates.

6.1.2.5 Confounding

Confounding is often described as a mixing of effects. A confounder is a factor that is associated with the exposure of interest, and is an independent risk factor for the outcome. Importantly, a confounder is not on the causal pathway between the outcome and the exposure. When confounding is present, the true association between the exposure and outcome is blurred due to the effect of the confounding factor. The effects of a confounder can be handled in several ways, for example by matching, randomization, restriction, stratification and in adjusted regression models²¹⁸. These methods require that potential confounding factors are considered in the study design, so that data on the confounding variable may be collected for participants.

Different methods of handling confounding have been used in this thesis. In studies I-III, reference individuals were *matched* for age at CD diagnosis, sex, calendar period at diagnosis of CD and county. In the Cox regression model, analyses were performed stratum wise and in the case-control studies, we used conditional logistic regression, thus accounting for confounding effects due to these variables. In studies I-III, we adjusted for confounding factors such as diabetes mellitus, country of birth (Nordic vs non-Nordic) (studies I-III), liver disease (III), educational level (studies II-III), and socioeconomic position (study I) by *inclusion in the regression model*. Additionally, we performed analyses *stratified* by age at CD diagnosis (study I), length of follow up (studies II-III), sex (studies I-III), and age and calendar period at first intestinal biopsy (studies II-III). To adjust for the potentially confounding effect of any previous renal disease, we used a model which we *restricted* to individuals without previous renal disease (study III). *Stratification* (univariate and bivariate analyses) and *inclusion in regression models* (main analysis) were methods used to assess potential confounding effects in study IV.

A common confounder in epidemiological studies is smoking. We did not have any data on smoking in our studies. Since smoking is a risk factor for VTE and ESRD, but has no association²²⁶ or a negative association²²⁷ with CD, it is unlikely to explain our findings in study I and II. A recent study shows no association between CD and educational level, however CD was slightly less common among individuals with low socioeconomic position²²⁸. We adjusted for socioeconomic position in study I, and for educational level in studies II-III. The protective effect of smoking and low socioeconomic position may both reflect an increased risk of undiagnosed CD due to different health-care seeking behavior among individuals with a low socioeconomic position.

6.1.2.6 Random error

Null results as well as positive findings may be due to chance. A random error reflects the power of a study, and will be minimized as the size of the population under study increases. The role of chance is roughly estimated by confidence intervals and p-values. A large sample size will result in a narrow confidence interval and a small p-value; we

say that the precision is high. It is important to distinguish between precision and validity. A high degree of precision does not imply a high degree of validity. When assessing an association between exposure and outcome, Hill's criteria of causal associations (see Table 8) should be considered irrespective of sample size and precision²¹⁸, bearing in mind that association is not equal to causation.

In studies I-II, exposure and outcome data were based on national registers allowing a large sample size and high precision. In study III, the outcome of study (IgAN) was rare yielding wider confidence intervals, non-significant results in stratified analyses and restricting the interpretation of stratified analyses due to strata with lack of positive events. Furthermore, the large number of subanalyses increases the risk of chance findings.

Table 8. Hill's criteria for causality.

- | |
|--|
| <ol style="list-style-type: none"> 1. Strength of the association 2. Consistency upon repeated observation 3. Specificity of the association 4. Temporality (cause preceding effect) 5. Dose-response or exposure-response effect 6. Scientific plausibility of the association 7. Coherence with available knowledge 8. Experimental evidence 9. Analogy |
|--|

6.1.3 External validity

The concept of external validity is defined as the extent to which the findings of the study are generalizable to other groups outside the population included in the study.

A threat to the external validity of study I is the limitation of CD to inpatients. As previously discussed, inpatient care was more common during the majority of the study period than it is today, and children (the majority of individuals in this study) more often require inpatient care during investigation for CD. Even today, some children may be hospitalized and require general anaesthesia for small intestinal biopsy acquirement. The population in study I is thus likely a mix of individuals admitted for therapeutic or diagnostic reasons, or for concomitant disease. The results of this study are not generalizable to individuals with potential or asymptomatic CD.

In studies II and III, external validity in *diagnosed* CD is high since we are confident that we have captured the vast majority of Swedish individuals diagnosed with CD during the study period. Our confidence in the data is further supported by findings of high specificity of CD in VA, and findings of high frequencies of small intestinal biopsies obtained by Swedish physicians when diagnosing CD¹²⁰. Again, since individuals with CD may be undiagnosed, these studies reveal no information regarding the risk of ESRD or IgAN in undiagnosed CD. Age at CD diagnosis could possibly be a proxy for the length of exposure of undiagnosed CD. In these studies, analyses stratified for age at CD diagnosis revealed no difference in risk of outcome according to age at CD diagnosis.

A potential issue for concern regarding generalizability of the studies in this thesis is the restriction to a Swedish population. Potential demographic differences, e.g. environmental exposures and genetics should be taken into consideration when extrapolating our findings to populations in other parts of the world.

6.2 FINDINGS AND IMPLICATIONS

6.2.1 Study I

VTE is a common source of morbidity and mortality¹⁶⁹. We hypothesized that individuals with CD would suffer increased risk of VTE, mainly due to the effects of chronic inflammation with hyperhomocysteinemia¹⁸². We found a modestly increased risk of VTE in adults diagnosed with CD. This association may in part be explained by surveillance bias.

We had no data on the adherence to a GFD in this study, nor data on follow-up biopsies. We can therefore not exclude that among the subjects under study, the risk of VTE may be higher in individuals with poor adherence and likely a higher degree of persisting intestinal inflammation.

Our results are in contrast with the previous study by Miehsler et al. that found no association between CD and VTE. That study was, however, smaller (CD n= 207), originally designed to study IBD and included only a small number of patients¹⁷⁰. A more recent, Danish register based case-control study examined the occurrence of CD in individuals with a record of VTE. Although no association was found (adjusted OR, 1.0; 95% CI 0.8-1.4), individuals with CD diagnosed within 90 days before VTE diagnosis were at increased VTE risk (adjusted OR, 9.2; 95% CI 1.4-61)²²⁹. Important differences between that study and ours is the inclusion of individuals with dermatitis herpetiformis in the CD group, inclusion of outpatients with CD, and adjustment for pregnancy or infection within 3 months prior to VTE diagnosis.

In conclusion, our study in conjunction with previous studies provide evidence that diagnosed CD is not a major risk factor for VTE.

6.2.2 Study II

Individuals with CD suffered a three-fold increased risk of ESRD. This risk increase was seen irrespective of age at CD diagnosis, sex, calendar period, and length of follow-up from CD diagnosis. Our results are coherent with previous research implying an association between CD and renal disease^{196 161, 195}. These studies were however limited to in-patients^{161, 195, 196}. Our results remained statistically significant after adjusting for diabetes mellitus type 1, excluding individuals with any prior renal disease, and excluding the first year of follow-up to minimize surveillance bias. When we stratified the analysis according to modality of treatment, we found an increased risk of dialysis but not renal transplantation in individuals with CD.

One must bear in mind the differences between absolute and relative risks when discussing the findings of this study. ESRD is a severe diagnosis with vast consequences in the lives of those affected. However, it is a rare disorder, and although we found a three-fold increased risk of ESRD in individuals with CD, the absolute risk of ESRD in CD was low (0.3%).

Biological mechanisms causing the association between CD and ESRD have yet to be explained, however shared disease characteristics such as increased gut permeability^{190, 191} and impaired oral tolerance^{192, 193} have been suggested.

This study has a few important limitations. We had no data on adherence to a gluten-free diet. We cannot exclude that increased inflammatory activity due to poor adherence could contribute to higher risk estimates. Furthermore, we had no data on smoking. Although smoking is a risk factor for ESRD, it has no association²²⁶ or a negative association²²⁷ with CD, and is unlikely to explain our findings.

Renal replacement therapy is associated with low quality of life, high mortality and morbidity, and high costs¹⁸⁶. The potential gains, for the patient and for the society, of preventing ESRD or delaying the need for renal replacement therapy in individuals with chronic renal disease are substantial. An important strength of the current study is that we included all individuals with diagnosed CD in Sweden. If others replicate our findings, clinical guidelines of CD may need to be altered to include greater awareness regarding renal function.

6.2.3 Study III

We suggest that individuals with CD suffer increased risk of IgAN. The risk was restricted to men, and stratified analyses had low power to detect significant risk estimates.

Our findings are in line with previous research. It has previously been shown that patients with IgAN suffer increased risk of CD¹⁹⁵. Although previous studies suggest an association between CD and renal disease, they do not provide detailed information on specific renal diseases^{161, 196, 205}. Although underlying mechanisms are unknown, the study by Collin et al negates association based on HLA genotype¹⁹⁵.

An important strength of this study is the classification of disease and exposure according to biopsy reports. Given the wide spectrum of disease presentation in IgAN, it is likely that the sensitivity of IgAN in this study is low (even though we presumably have included all cases of *diagnosed* IgAN). Furthermore, IgAN is inseparable from Henoch-Schonlein Purpura upon biopsy examination¹⁹⁹. We assume that written referrals include patient histories and that this is taken into consideration by the pathologists when diagnosing IgAN. We know of no validation studies of the IgAN diagnosis in Swedish biopsy registers.

We know of no previous study investigating the risk of biopsy-verified IgAN in biopsy-verified CD. In conclusion, men with CD suffer increased risk of IgAN. This may contribute to the observed association between CD and ESRD.

6.2.4 Study IV

Although we hypothesized that infection at time of gluten introduction would increase the risk of future CD, we found no such association after adjusting for age at gluten introduction, age at end of breastfeeding, and age at infection. Nor did we see an association between age at gluten introduction, or age at end of breastfeeding and risk of future CD. This contradicts previous research, that has found increased risk for CD

among children with short breastfeeding duration < 2 months⁸⁸, < 90 days⁸⁴ or < 30 days⁸⁷, although results have been inconsistent^{89, 90}.

We did not have any data on clinically diagnosed infectious disease or specific pathogens but only parent-reported infectious disease. This is a disadvantage, and may lead to misclassification of exposure. The lack of detail regarding aetiologies may blur existing associations between certain pathogens and risk of future CD. Furthermore, we have no data on gluten dosage, which might impact the risk of an early CD diagnosis.

An important feature of the current study is its prospective design. Additionally, children included in the ABIS study were born in 1997-1999, that is *after* the Swedish epidemic of CD, and after the introduction of new infant dietary guidelines by the Swedish Pediatric Society (see Figure 3). More than one half of all children were breastfed for > 9 months. Gluten was introduced in months 5-6 in the majority of children. No children with CD were exposed to gluten before the age of 2 months. The changes in infant feeding pattern seen in Sweden the past decades⁵⁸ may explain why we did not find an independent effect of breastfeeding nor age at gluten introduction on the risk of future CD, whereas previous studies did^{85, 86}. We conclude that given current dietary guidelines, these environmental factors are no major risk factors for CD.

6.3 IMPLICATIONS FOR FUTURE RESEARCH

Future research should consider:

- The potential effects of GFD adherence on the occurrence of subsequent complications.
- Increased knowledge regarding mechanisms and combinations of risk factors that lead to complications. Such awareness could possibly allow for identification of individuals at risk. In a visionary future, where health care is increasingly personalized, individual parameters may aid in determining individual risk-profiles and disease management strategies.
- Further studies of individuals with ESRD, to compare disease characteristics in patients with and without CD. Thereby it would be feasible to determine possible unique features in terms of survival, benefit from various cardiovascular treatments, and parameters such as anemia and bone metabolism in patients with CD and ESRD.
- Validating the IgAN diagnosis in Swedish biopsy registers.
- Specify pathogens when assessing the role of infection in CD pathogenesis.
- Including longer follow-up periods in studies of infant feeding patterns and future CD, to determine whether these factors merely affect induction time, truly protect against CD or have no association with CD.

7 CONCLUSIONS

I) CD is associated with a modestly increased risk of future VTE. This risk increase is restricted to individuals diagnosed with CD in adulthood, and is likely the result of a combination of chronic inflammation and surveillance bias.

II) Individuals with CD suffer a three-fold increased risk of future ESRD, independent of age at CD diagnosis. We suggest awareness regarding renal function in individuals with CD.

III) Individuals with CD suffer increased risk of IgAN. This risk increase was restricted to men and we had low power to conduct stratified analyses. IgAN may be an underlying contributor to the increased risk of ESRD seen in CD.

IV) Children with infection at time of gluten introduction suffer no increased risk of future CD. Contrary to previous research, we did not find an independent effect of breastfeeding duration nor age at gluten introduction on the risk of future CD.

8 SAMMANFATTNING PÅ SVENSKA

Celiaki (glutenintolerans) är en sjukdom som drabbar cirka 1% av befolkningen i västerländska populationer. Hos individer med celiaki leder intag av vete, råg eller korn till en immunmedierad inflammation i tunntarmen och destruktion av tarmluddet. Celiaki kan debutera när som helst i livet. För att drabbas av sjukdomen krävs att man har generna HLA-DQ2 eller HLA-DQ8. Eftersom denna genotyp är vanlig (återfinnes hos cirka 1/3 av befolkningen) så kan den inte förklara varför vissa individer får celiaki. Mekanismerna bakom sjukdomsutvecklingen i celiaki är okända, dock har tidigare forskning pekat på att amning kan skydda mot celiaki, och att viss infektionssjukdom är vanligare hos de som drabbas av celiaki. Individer med celiaki löper ökad risk för ett antal andra sjukdomar, exempelvis diabetes mellitus typ 1, thyroideasjukdom och depression.

Syftet med denna avhandling var att identifiera tillstånd och komplikationer som är kopplade till celiaki, för att bättre förstå sjukdomen och kanske kunna förebygga komplikationer, samt eventuellt identifiera grupper med förhöjd risk för celiaki. Ett annat syfte var att undersöka huruvida risken för celiaki påverkas hos barn som lider av infektion samtidigt som de för första gången i sitt liv äter mat innehållande gluten.

I studie I undersökte vi risken för venös tromboembolism (VTE; lungemboli eller djup ventrombos) hos individer med celiaki. Tidigare forskning har föreslagit att den kroniska inflammation som föreligger vid celiaki leder till hyperhomocysteinemi, vilket i sin tur är kopplat till förhöjd risk för VTE. Vi identifierade 14,207 individer med celiaki i det svenska slutenvårdsregistret, och 69,048 matchade kontroller i befolkningsregistret. Genom Cox regression fann vi att individer med celiaki löper en lätt förhöjd risk för VTE (Hazard ratio, HR, 1.86; 95% konfidensintervall, KI, 1.54-2.24). Sambandet mellan celiaki och VTE sågs endast hos individer som fått sin celiakidiagnos i vuxen ålder (>15 år). Vi tror att denna överrisk beror på kronisk inflammation, men också till viss del på grund av ett skevt urval då vi endast studerade patienter med celiaki som slutenvårdsdiagnos.

Terminal njursvikt innebär ett behov av njurtransplantation eller kontinuerlig dialysbehandling. Tidigare studier har föreslagit en koppling mellan celiaki och terminal njursvikt, men har varit baserade på celiaki diagnosticerad i slutenvård vilket ger risk för en skev bild. I studie II identifierade vi svenskar med celiaki genom deras tunntarmsbiopsi. Samtliga patologikliniker i Sverige kontaktades och rapporterade individer med celiaki från 1969-2008. 29,050 individer med celiaki och utan njursvikt vid studiens start identifierades, liksom via befolkningsregistret 144,363 matchade kontroller. Terminal njursvikt identifierades initialt genom koppling till sluten- och öppenvårdsregister samt senare även till Svenskt njurregister. Vi fann en nästan tre gånger förhöjd risk för terminal njursvikt hos individer med celiaki (HR, 2.87; 95% KI 2.22-3.71). Risken var något högre när vi begränsade utfallet till individer med njursviktsdiagnos i ett patientregister *och* i Svenskt njurregister.

IgA nefropati är en njursjukdom som karaktäriseras av njurinflammation och depositioner av IgA antikroppar i njurens mesangium. Den drabbar cirka 15-40

personer per miljon människor och år och leder hos 15-50% av fallen till terminal njursvikt. I studie III undersökte vi risken för IgA nefropati hos personer med celiaki. Detta då en tidigare studie funnit en ökad risk för celiaki hos patienter med IgA nefropati, men även för att bättre förstå vad som orsakar kopplingen mellan celiaki och njursjukdom. Vi utgick ifrån samma celiakicohort och referenspopulation som i studie II. För att identifiera dem som utvecklat IgA nefropati kontaktade vi de fyra patologikliniker som diagnosticerar njurbiopsier. Genom datoriserade sökningar fann vi 4,069 individer med IgA nefropatidiagnos i svenska biopsiregister. Individer med celiaki löper en förhöjd risk för IgA nefropati (HR, 3.03; 95% KI 1.22-7.56). Riskökningen sågs endast hos män.

I studie IV undersökte vi mekanismerna bakom sjukdomsutveckling i celiaki. Vår hypotes var att en infektion under den känsliga tidpunkt i livet då barn för första gången inkluderar gluten i kosten leder till ökad risk för celiaki, förslagsvis på grund av felaktig aktivering av immunsystemet. Detta undersökte vi med hjälp av data från ABIS studien (Alla Barn i Sydöstra Sverige); en studie som genomfördes 1997-1999 för att undersöka miljöfaktorer relaterade till immunmedierad sjukdom. Föräldrar till barn som föddes under studieperioden erbjöds delta. Studiedeltagare erhöll en första enkät på BB, och ombads sedan föra en dagbok över barnets sjukdomar och födointag under första året. Av de 21,700 barn som föddes, valdes föräldrarna till 17,055 barn att delta. Slutligen erhöll vi komplett dagboksdata (med information om amningslängd samt datum för glutenintroduktion) för 9,408 barn varav 44 utvecklade celiaki. Information om celiakidiagnos hämtades från barnklinikerna i området. Hos dessa barn förekom 42,826 föräldrapporterade infektionsepisoder. När vi justerade för amningslängd och ålder vid infektion och glutenintroduktion, fann vi att infektion vid tiden för glutenintroduktion inte är kopplat till en förhöjd risk för framtida celiaki (HR, 1.8; 95% KI 0.9-3.6). Gastroenterit vid glutenintroduktion var ej heller kopplat till risk för framtida celiaki (HR, 2.6; 95% KI 0.2-30.8). En nackdel med denna studie är att vi endast kunde följa barnen fram till och med 30 juni 2006. Det är därför möjligt att de parametrar vi studerade endast förskjuter symptomdebut längre fram i livet. Vidare är det möjligt att specifika patogener, exempelvis rotavirus, påverkar celiakirisken, vilket vi ej kan utesluta i denna studie.

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